

Reform by Inches or Half-inches

It is still an open question whether the British Association for the Advancement of Science will manage to survive the years ahead. The steps taken at the Swansea meeting of the association two weeks ago (see *Nature*, 233, 80; 1971) to modernize the administration of the association are steps in the right direction, but they are manifestly small and even timid steps. There is a chance that the association's management might have moved even more cautiously if it had not been for the unaccustomed toughness of the voluntary committees, sixteen or so of them, which are responsible for the detailed arrangement of the programme of the association's annual meeting. From all appearances, even the heart-searching of the past year, which has at least seen the production of a fearless analysis of the association's financial and intellectual plight (see *Nature*, 231, 412; 1971), seems not to have created a sufficient sense of urgency. The result is that the Swansea meeting was presented not with a tangible programme of reconstruction but with an invitation to appoint yet another committee to put flesh on the bones of the proposals put forward by the Committee of Review three months ago. The danger, of course, is that the association's claim on public attention, like its investment income, is being drained away with the passage of each week or month.

Why is the association making such heavy weather of its attempt to stay in business? Gone, it seems, is the robust independence of the early nineteenth century, when the British Association came into being (in the year in which the First Reform Bill was being fought through the British Parliament) because its founders recognized the need of an independent and even irreverent forum for interdisciplinary discussions. (Those were bad times for the Royal Society, which might otherwise have done the job.) Only a part of the reason lies in the intricacy of the association's constitution, with its complicated hierarchy of committees intended chiefly to ensure that responsibility lies nowhere identifiable. There is also a serious danger that the association is quite unreasonably imprisoned by conventions which belong to the second half of the nineteenth century, not the first.

The pomp and ceremony which attends each annual meeting, and which must leave a good many of those who went to Swansea hanging their heads in shame, is one obvious sign of this symptom of the association's trouble. Is it really necessary that the first item on each year's programme, increasingly intended to be a means of provoking public discussion of important issues, should consist of a solemn procession in which academics and would-be academics dress up in academic finery and make their way like actors in a bad Greek chorus through a public auditorium to a platform more often used by actors than by participants in a ceremony for awarding

honorary degrees? Is it really in keeping with the association's wish to perform a real service in the modern world that there should be yet another procession to the local cathedral to hear some bishop deliver himself of familiar pronouncements on the place of science in society at large? And even if good fellowship (of which there is plenty) must be acknowledged to be an important part of the association's annual rites, is it really necessary that there should be so many invitations for men and women to dress up in evening clothes? Harmless though these conventions may be in ordinary circumstances, an organization seeking to make a mark on public opinion as well as on the opinions of its members must necessarily use its public ceremonies as ways of reinforcing what it has to say. Especially because it is a long time since the association had constructive and sharp things to say about issues of public importance, nobody should be surprised that it has acquired a public reputation much more appropriate to a snobbish and old-fashioned club than to an organization seeking to enhance the public reputation of modern science in a modern and sceptical world.

What is to be done? The first need is that the association should form a clear vision of what it wants to be. If, as seems entirely proper, one important objective is the encouragement of an informed opinion on public issues with scientific antecedents or connexions, the association will have to work out ways of defining what these issues are and what needs to be said about them. This is intellectual work, not simply administration, which means that devices will have to be developed for containing controversy within the framework of the association. If the association is to play a creative part in the encouragement of European collaboration in science, as Professor W. H. McCrea and Sir Brian Flowers (see page 173) have been urging, then it will have to become more professional in organization and more imaginative in innovation. If the association wishes, as it should, to go on providing stimulating intellectual fare for those who turn up at its annual meetings, there is a case for strengthening, not for eroding, the voluntary organization of the association into sections of specialists which has provided the pattern of the past century. It is a great surprise that the Committee of Review, responsible for the past year's self-examination, has done so little to follow up the suggestion that stronger links should be forged between the British Association and the more conventional learned societies.

But is it worth the trouble? This is what a great many British scientists will be asking. That they do so is a measure of how, over the years, the association has lost its hold on the interests and affections of professional scientists in Britain. Luckily, however, there is still enough support to ensure continuity, at least for another year or two. Moreover, it is now more clear than ever



that there is a public need for what the association might accomplish. Rarely has the profession of science been as widely misunderstood and wrongly maligned than at the present time; the British Association could help enormously to set the record straight, not so much by means of counter-propaganda as by the objective study of important public issues on which public opinion seems commonly to founder. This is a task which is almost by definition beyond the scope of the orthodox societies. It

is also one that cannot be left to an organization such as the British Society for Social Responsibility in Science—that has become such a vigorous pressure group that it cannot be a sounding board for informed opinion at the same time. It is earnestly to be hoped, in the critical months ahead, that professional scientists in Britain will recognize that the fate of this distinguished and potentially valuable institution will depend on their help—or at least on their indulgence.

Opportunities for Collaboration in Europe

IN spite of all the enthusiasm for European collaboration that has become common in the sphere of the British Government's influence, very little has been done to show how the institutions responsible for the support of scientific research should be adapted to meet the future needs. Although the imaginative declaration by Sir Brian Flowers at the British Association last week (reprinted in full on page 173 of this issue) is necessarily a first attempt to list the jobs that must at some stage be done, it is encouraging that serious public discussion has at least begun. The question now is what should be done to advance the cause, and to make the prospect of European collaboration a reality. It goes without saying that the first essential is to recognize that in the long run, the creativity of the European scientific community is potentially of inestimable value.

The first thing to be said is that European collaboration in science will not yield its full potential until the university systems of Western Europe are much more closely integrated with each other than at present. There is no point in beating about this bush. At some stage, it will be necessary to ensure that centres of research will be easily accessible to all who work in their fields of excellence. In many cases, this implies that universities should be able to appoint to their faculties people of foreign nationality simply on the strength of their potential contribution to research and teaching. Elsewhere, research institutes will have to be similarly accessible. There are obvious difficulties when systems of higher education are administered as if they were parts of the government, as in France or Italy, but it will probably be much more difficult to arrange that research institutes, most of which are supported by public money, will be similarly accessible. When, for example, will the *Centre Nationale de la Recherche Scientifique* be allowed by the government of France to take on its payroll scientists from elsewhere? In the same spirit, when will the British government agree that the Agricultural Research Council (if it survives the report of the Rothschild Committee) should employ scientists from France or the Netherlands? The Commission of the European Communities has made some progress, in the past few months, towards the definition of rules for regulating the transfer of professional qualifications from one European country to another, but this is a far cry from the arrangements that will eventually be necessary for allowing foreign participation in institu-

tions which are traditionally regarded as national in their essentials.

Beyond that hurdle is the problem of what should be done to rationalize—"to harmonize" as they say in Brussels—the pattern of basic research in Europe. In the past few years, there have been notable successes in collaboration in fields such as high-energy physics, and there is no doubt that CERN is a model of how nations can function in such a way that their collective efforts are more valuable in combination than separately. It is only proper to recall that in the past few years, there have been failures as well as successes. Sir Brian Flowers has done a public service in asking that schemes for collaboration should be designed in a spirit of realism and not with the hope of extraneous benefits. The failure of ELDO shows what is likely to go wrong when institutions are designed to bring political rather than technical benefits, but it is also a great misfortune that, more recently, European nations have made very little headway in collaboration on the construction of large telescopes. But if a sensible coordination of plans is as difficult as this in fields in which there are expensive and conspicuous instruments to be built, what prospect is there for cooperative planning in less spectacular fields of inquiry?

The obstacles are obvious and formidable. The past few years have shown how carefully the Science Research Council in Britain has had to move in its attempts to mould the pattern of research at British universities. It is easy enough to persuade universities and other research organizations to accept that there can be only a few centres of excellence in any field, but the same institutions, however enlightened, are usually unwilling to accept that laudable attempts to concentrate research at selected centres should deprive them of what they consider to be their rights. Some countries on the mainland—France, for example—are better able to work out patterns for the conduct of research because of their centralized systems of support, but elsewhere—as in West Germany—the machinery for the channelling of public money to basic research is an awkward blend of centralized direction and decentralization, with the universities only accidentally involved in the conduct of research. In short, European institutions for the administration of the public money spent on basic research are such an inhomogenous group that structural considerations will impede coordination. To begin with, the best that can be hoped for is that the

governments of Europe will establish some kind of talking shop within which research councils, public research foundations and universities can begin to work out what needs to be accomplished. Quite independently of whether the European Community is to be enlarged, this is a task that could be attempted right away.

Further away is the problem of how money should be spent on European research. For the time being, at least, it is to be expected that the member nations of the European Community will regard what they spend on higher education and basic research as a long-term investment in national well-being or even prosperity. Their justification for this expenditure will often rest on the belief that such expenditure will improve their competitive position within the communities. With luck, in other words, natural rivalry should help to ensure that European governments spend generously on higher education and

basic research in the decade or so ahead. But this is bound to be a passing phase. As soon as there emerges a rational pattern of research, it will also be necessary to work out ways of coordinating expenditures of funds. That will be the point at which governments with large budgets for research and higher education will be looking askance at those who drag their feet. At the same time, it will be necessary for governments (and taxpayers) to accept that national resources should be spent on foreign enterprises. At this stage, it is hard to see how governments can be prevented from tearing each other to pieces without an international budget for research and higher education. The fact that such a goal is bound, at present, to seem distant should not allow those who administer public expenditure on research and development to shy away from the fact that the planning must be done now, and not shelved until some future date.

From Prayer-meeting to Trade Fair

THE fourth United Nations Conference on the Peaceful Uses of Atomic Energy, now finishing at Geneva, is a remarkable proof of how rapidly it has been possible to convert a bright idea into a workable and sophisticated technology. It is also a powerful demonstration of the way in which technological innovations should be introduced to those most likely to benefit from them with a greater sense of sobriety than that attending the first United Nations conference in 1955. Will the sponsors learn the lesson now staring everybody in the face?

Fifteen years ago, nuclear power was still largely military in character. The first United Nations conference was remarkable for the way in which it helped the governments of the nuclear powers to put some of their secret cards on an open table. Even such facts of nature as the cross-sections of nuclear reactions involving neutrons were secret, and it seemed at the time to be a great discovery for all concerned that measurements on both sides of what was then called the Iron Curtain tended to be roughly in agreement with each other. To be sure, it was at the time a puzzle to know just why the nuclear powers had decided to make a partially clean breast of the information which they had acquired in the pursuit of military objectives. Perhaps they were responding honestly to the complaint of non-nuclear nations that it would be intolerable if the Great Powers were able to hang on to what seemed to be a monopoly in the exploitation of an important and beneficial technology. It may be that the initiative of President Eisenhower, who launched from the United States an international programme with the slogan "Atoms for Peace", struck a genuine response from the Soviet Union and the United Kingdom. It may even be, as the sceptics were inclined at the time to protest, that all concerned had their eyes on the main chance—the opportunity for making important sales of equipment. In any case, there is in retrospect no doubt that the promise of nuclear energy was spelt out with too much enthusiasm, and that in the process the development of the industry was hindered and not helped. The moral is that in the launching of a new technology, as in the launching of a business enterprise, it is imprudent to publish too cheerful a prospectus.

A second lesson to be learned from the past two weeks at Geneva is that technical progress in nuclear power has been as rapid as anybody could realistically expect. Fifteen years ago, even thermal reactors were entirely novel. Only the foolhardy talked seriously of fast reactors. Although there has since been all kinds of false excitement about fusion reactors, it does now seem as if thermonuclear power is about to be a reality. The importance of this development cannot be exaggerated—it is tantamount to freedom from anxiety about energy supplies in the foreseeable future. That makes up for all kinds of errors.

100 Years Ago



THE preparations made by the Governments of the present age to have every phase of a total eclipse studied and recorded, contrast favourably with the superstition that prevailed a few centuries ago. For instance, the *Scientific American* quotes the following from a German paper:—"The Elector of Darmstadt was informed of the approach of a total eclipse in 1699, and published the following edict in consequence:—'His Highness, having been informed that on Wednesday morning next at ten o'clock a very dangerous eclipse will take place, orders that on the day previous, and a few days afterwards, all cattle be kept housed, and to this end ample fodder be provided; the doors and windows of the stalls to be carefully secured, the drinking wells to be covered up, the cellars and garrets guarded so that the bad atmosphere may not obtain lodgment, and thus produce infection, because such eclipses frequently occasion whooping cough, epilepsy, paralysis, fever, and other diseases, against which every precaution should be observed.'"

OLD WORLD

BRAIN DRAIN

The Circus Leaves Town

QUIETLY, without any fuss, the North American Joint Selection Board has ceased to exist due to the withdrawal of the Civil Service Commission (CSC). The board, comprising representatives of the CSC, the United Kingdom Atomic Energy Authority (UK AEA) and the Central Electricity Generating Board (CEGB) toured North America to recruit the best of the young British research scientists and engineers who had emigrated as part of the Brain Drain, and wanted to return.

Established in 1958 (the CEGB joined in 1962) under the chairmanship of Mr Harry Hoff, the board—which became known to applicants as Hoff's Circus—saw its job not as a mission to reverse the Brain Drain but as an attempt to recruit the best people to carry out research work in Britain.

The Civil Service Commission has now withdrawn because it feels that the operation "is no longer cost effective" and because at the moment it is finding "the calibre and quantity of recruits required" in Britain. As the costs do not diminish proportionately because one of the parties has withdrawn, the UK AEA and CEGB have decided not to continue. Costs have risen rapidly despite attempts to cut them to a minimum by introducing a greater degree of preselection of candidates. In the board's 1968 report it was estimated that the cost was "about £500 per man recruited—scarcely any more, if not less, than the cost of recruiting a comparable man in the United Kingdom". The exact figure for last year is not known, but it was in the region of £1,000–£1,200 per man.

The UK AEA and CEGB are not particularly distressed to see the board go, although both would have been happy for it to continue. With post-graduate jobs hard to come by both are getting plenty of applicants. On the other hand, whereas there is no suggestion that all the brightest graduates go abroad, there is no doubt that the standard of the recruits from America, with their extra year or two of experience was very high. Sixty or

seventy per cent of those interviewed were recommended for appointment but as can be seen from the table less than 20 per cent were actually placed.

Until recently it looked as if the board's activities were about to expand. The Post Office was interested in joining and for the past two years information about suitable applicants has been passed on to British Rail and the British Steel Corporation.

But if the board's members no longer need recruits from America, there are still many British subjects in America who would like to return home. The number of applicants has been increasing over the years and Management Selection Ltd (MSL), who provide information and advice to both applicants and employers, say that, if anything, there are more scientists wanting to return. The problem for the exile is that whereas one interview with the board allowed him to be considered for several posts in Britain, he now has to come to this country at his own expense in the hope of finding a job when he gets here. It is ironic that just when the employment situation for highly qualified scientists in this country is

very difficult, one of the organizations that made it easier to find a research job has ceased to function.

Companies (and the board members) are perfectly willing to consider the exile for research posts in Britain, but recently the demand for highly qualified people has fallen away, and the companies are finding that by and large they, too, are finding the quantity and quality they want in Britain, and only when they are sure that an applicant from North America is suited to the post are they prepared to pay the fare for the applicant to attend an interview in Britain.

Now that the Brain Drain is no longer a subject for popular concern it is interesting to note that with the employment situation for PhDs becoming increasingly difficult in North America (see *Nature*, 233, 87; 1971) more Americans are inquiring at MSL about job possibilities in Britain. Seems like the end of a chapter.

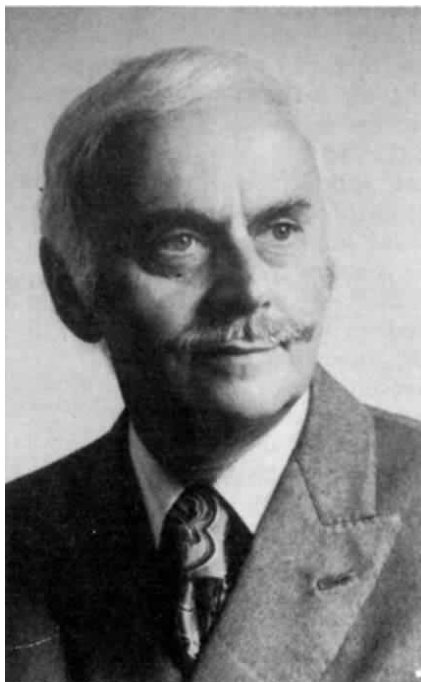
MOLECULAR BIOLOGY

EMBO not for Culham

THE decision by the British government not to offer Culham as a site for the proposed European Molecular Biology Organization (EMBO) laboratory last week caused less surprise in scientific circles than it did in the ranks of the Oxfordshire County Planning Committee.

The decision is only one more chapter in the lengthening history of the laboratory. Although conceived in 1963 when EMBO was first formed, no final decision has yet been taken on whether the laboratory will be built but this is expected to be recommended by an international conference next month.

Britain is only one of the countries to consider offering a site for the laboratory and the eventual decision not to do so was taken according to the Department of Education and Science, because "the sites being proposed by West Germany offered better facilities and climate" than the Culham site. In the 1967 proposal for the laboratory it was stated that the site must be in distinguished intellectual surroundings, be easily accessible, have good amenities in terms of climate, housing, international schools, language and countryside, and the revised proposals in 1970 emphasized the need for the laboratory to be near a large research centre in which sophisticated instrumentation and computation are being developed for other purposes. Mr Hugh Farrant, chairman of the Oxfordshire Planning Committee which had welcomed the proposal of Culham as the site last year, claims that apart from the stipulation about climate, the Culham site fills the bill, having ade-



Photograph by Mark Gerson, FIIP, ARPS

Mr Harry Hoff of the UK AEA, Chairman of the North American Joint Selection Board throughout its thirteen year life.

Success of the North American Joint Selection Board 1966-71

Year	Applied	Interviewed	Placed
1966-67	—*	215	39
67-68	—	249	47
68-69	—	257	36
69-70	250	173†	30
70-71	302	186	>25‡

* Figures not known

† Preselection introduced

‡ Final figure not yet known

quate communications and being close to both Oxford and Harwell.

The considerations that led to the decision probably included the fact that Germany is prepared to put considerably more money into the project than any of the other governments involved and has two suitable sites to offer, at Munich and Heidelberg, which are both in the centre of Europe. A further consideration is that Dr John Kendrew of the Medical Research Council's Molecular Biology Laboratory at Cambridge is the likeliest candidate to become the first director of the proposed laboratory and a British director of a European laboratory in Britain would not appear very European.

Mr Airey Neave, MP for Abingdon, who described the decision as "rather disappointing" is to ask Mrs Thatcher, Secretary of State for Education and Science, for a full statement, but as the decision was taken after full consultation with EMBO officials it is unlikely to provide ammunition for the dissenters.

COMMUNICATIONS

Dial Around the World

THE largest single contract ever awarded by the British Post Office has been given to Plessey Telecommunications for the first stage of Britain's newest international telephone exchange. The exchange will use crossbar switching and will provide more than a third of the 18,000 international telephone circuits eventually planned to be handled by the Post Office's new Mondial House, now under construction. As part of the contract, worth about £10 million, Plessey will also provide a complex of computers which will perform the complicated accounting procedures involved in international telephony—the cost of each call must be apportioned among the countries of its origin, its destination and its passage. The same equipment can, at the same time, give precise information on how the exchange is routing the calls presented to it.

For the Post Office, the opening of the exchange in Mondial House at the end of 1973 will mean a larger increase in the number of destinations to which British telephone subscribers can dial their own telephone calls. Until this year, the Post Office depended on its exchange at Faraday House, with its 2,000-circuit capacity to handle all international calls. The Wood Street international exchange has now begun to operate and will eventually have a capacity of 8,400 circuits. But when Mondial House's 18,000 circuits are available, there will be connexions, by means of cable, microwave and satellite, to 51 countries, including 29 in

Europe as well as the United States, India and Australia. As the number of countries which can dial each other increases, thanks largely to satellites, Britain's importance as a transit centre will increase—and so will the Post Office's revenues from external telecommunications.

For Plessey, the award is a reminder that the Post Office has fully repented of its error of the 1950s when it vetoed the crossbar switching system with which the United States, Sweden, Japan and some continental countries were modernizing their telephone networks. Plessey (or the company which it has since absorbed, Automatic Telephone and Electric) developed crossbar privately, without a Post Office subsidy. Only in recent years has Plessey persuaded the Post Office to adopt it as a standard exchange.

Plessey has also developed the newer kind of exchange described as electronic but actually based on reed relays—which the Post Office is also installing. The system is called TXE-2 and the Post Office eagerly publicizes the gradual introduction of electronic exchanges into the telephone network. But Plessey executives last week made it plain that only crossbar, and not the much-publicized TXE-2 switch, could handle a telephone exchange of the size and complexity required by Mondial House.

Considering Plessey's position as British developer of crossbar it was unlikely that the contract would go to the Post Office's other major suppliers, GEC-AEI and Standard Telephones and Cables. Plessey can probably be fairly confident of supplying the rest of the Mondial House exchange. But the Post Office may not always buy British. It is showing a new boldness in accepting foreign suppliers and gave Philips of Eindhoven a much-prized contract for an international telegraph switching exchange just a few weeks ago. Plessey received this big Mondial House contract fairly easily but neither it nor the other two manufacturers can afford to be complacent about the future.

NUCLEAR PHYSICS

Cause for Concern?

CONCERN is growing among nuclear structure physicists in Britain that the proposed national nuclear structure facility will absorb all the finance available for nuclear structure research and that the facilities now available at the universities will be declared obsolete with a consequent withdrawal of Science Research Council support.

Sir Brian Flowers, chairman of the Science Research Council, said last week that when the national facility is built

all other accelerators in the country will be outdated and that support would be withdrawn from several of these facilities in order to concentrate on the new machine. In particular, Sir Brian mentioned that the Heavy Ion Linear Accelerator at Manchester University and the Tandem Van de Graaff at Liverpool University would be among the first to have their support cut whereas the most recent accelerators at Oxford University would still be supported. It would all depend, he said, on the nuclear structure budget at the time at which the national centre became operational. Sir Brian emphasized that the matter is now in a state of flux and that nothing definite would emerge until a decision was taken on the national facility; this will probably be in eighteen months' time when the design study team, at present at work at Daresbury, have completed their work (see *Nature*, **232**, 76; 1971).

K. W. Allen, professor of nuclear structure at Oxford University, said this week that it was unreasonable and unthinkable that support for the Oxford accelerators would be withdrawn and that such a decision would be contrary to current policy. The Oxford accelerators are at present supported to the tune of £250,000 a year, but there is no question of this being withdrawn before 1976, the earliest date for completion of the national facility if the green light is given soon.

Scientists at Liverpool are also concerned about the future of their laboratory but they are understandably less worried than the Oxford physicists. Although no final decision has been taken by the SRC on the siting of the national facility, the fact that the design study team is sited at Daresbury tips the scales in favour of a site in the north of England. Daresbury is within easy reach of both Liverpool and Manchester and if funds are withdrawn from both these universities they can still effectively carry on their research. But the position of the Oxford physicists and others farther away from the site is not so cheerful.

Enthusiasm for the nuclear structure centre will undoubtedly wane in some quarters if, on completion of the design study, there is a possibility of closing down all other facilities in favour of the new centre. If this happens, future developments in nuclear structure in Britain will have to turn in some other direction. One possibility is the upgrading of the Oxford accelerators by replacing the 12 MeV tandem Van de Graaff with a more powerful version (see *Nature Physical Science*, **232**, 76; 1971). Such a proposal is under consideration and if the SRC supports this improvement it will possibly signify the beginning of the end for the National Nuclear Structure Facility project.

TECHNOLOGY

Innovation Research

THE smaller member countries of the Organization for Economic Cooperation and Development, such as the Netherlands, Sweden and Switzerland, have been very successful in reaping the benefits of technological innovation. That is one of the conclusions to be drawn from an extensive international review of research on the processes and mechanisms of technological innovation published recently by the OECD (*The Conditions for Success in Technological Innovation*, OECD, Paris; 1971).

The aim of the report is partly to assess how member countries of the OECD have measured up to technological challenges and partly to discover how an increasingly favourable climate for innovation can be created. Some of the conclusions of the report, such as the idea that industrial competition is vital to successful innovation, are, however, still matters of contention which are unlikely to be resolved because of a lack of data. More than half of the 155 accounts of research cited in the report are concerned solely with the United States and the report calls not only for more research in other member countries of the OECD but also for further investigation of particular aspects of the problem, such as the mechanisms of technology transfer between industries and the time scales involved in innovation processes.

The OECD report follows closely on the heels of a British report by the Centre for the Study of Industrial Innovation (see *Nature*, 232, 432; 1971) which analysed the reasons for the abandonment of technological projects; before that, for example, there was the report of the Charpie committee to the US Department of Commerce which highlighted, possibly to an unrealistic extent, the importance of small companies in the innovative process.

The latest study is intended as a sequel to the series of reports called *Gaps in Technology* and published by the OECD during the past few years. The innovation process is considered from the point of view of industry, government and the universities—the three principal components of the framework in which innovation takes place. Important relationships seem to exist between companies of differing sizes because, for example, small companies tend to specialize in sophisticated technological areas which require smaller production capacity but which often make use of the experience of scientists and engineers who once worked in large industrial (or government) laboratories. The mobility of scientists should be encouraged, says the report, because it accelerates the diffusion of technology and brings fresh

minds to bear on particular problems. Industrial management also faces many problems of adjustment to an ever-changing situation but these can be overcome if entrepreneurial attitudes are encouraged, not only in individual managers but also in whole companies and institutions so that information can be exchanged easily across organizational boundaries and changes made

quickly and efficiently as the situation demands.

The report also suggests that governments can contribute to the improvement of the innovation process through changes in the tax and patent systems and that government procurement will continue to play a vital role in improving the technological prowess of industry.

Miscellaneous Intelligence

FEARS for the fish stocks in the North Atlantic will be strengthened by the reports of experimental trawling carried out in the sixties by the Fisheries Research Board of Canada (Technical Report 260). Operations on the Scotian Shelf, south-west of Nova Scotia, have been systematic enough in the past few years for accurate estimates of fish stocks to be possible. The Biological Station at St Andrews, New Brunswick, estimates that the standing crop of cod on the Scotia Shelf amounts to 464 million pounds. Argentine, pollock and haddock come next in order, with standing crops of 453 million pounds, 428 million pounds and 391 million pounds respectively. In case this should seem plenty of fish for all the trawlers now operating in the region, however, it is relevant that the laboratory's estimate of the "maximum sustainable yield" of cod—140 million pounds a year—is already less than the sum of the commercial landings of cod each year, 190 million pounds or so. In the circumstances it is forgivable that the report should draw attention to the largely unexploited stock of squid on the Scotia Bank, enough to provide 121 million pounds of edible or nearly edible food a year.

ASLIB is about to take over from the Department of Education and Science the thankless task of trying to maintain an advance list of international scientific conferences. Nobody will begrudge these tireless librarians the £8.00 which they propose to charge subscribers for their quarterly list, but it will be interesting to see precisely how Aslib tries to solve the awkward problem of separating international conferences proper from those to which the organizers have invited a few ritual foreigners, sometimes in the hope that the hospitality will be repaid.

THE first full-scale nuclear power station to be built in Sweden, the 440 MW boiling water reactor plant at Simpevarp on the east coast, supplied electricity to the Swedish grid for the first time last month. Its output is expected to reach 350 MW by the end of this year and

full capacity will be achieved during 1972. Most of the construction work was carried out by the Swedish mixed state-private company, ASEA-Atom, which has developed a commercial boiling water reactor capability independently of the United States. A second nuclear power station (580 MW) is expected to commence operation in 1974. During the 1980s, the construction of a further four nuclear power plants is planned and the total nuclear generation capacity should then be about 5,000 MW.

A NEW research vessel for the Natural Environment Research Council, RRS Challenger, was launched last week. The Challenger is the first Council vessel to be purpose-built for research, the other eleven in the NERC fleet have all been converted; she will operate from the council's research vessel base in Barry, Glamorgan. RRS Challenger has a displacement of 1,440 tons but is not the largest of the fleet. The new vessel has accommodation for nine scientists and is equipped for marine, geophysical and other oceanographic research. The Natural Environment Research Council expect the Challenger to be operational by April 1972 and most of her cruises are expected to be in the north Atlantic.

"To conserve is the Museum's first priority; to educate and entertain is a close second. To conserve for future generations whilst ignoring the present generation would be absurd", is one of the opening remarks of *Museums in Education*, Education Survey No 12, HMSO, 50p, published this week. The survey conducted by Her Majesty's Inspectors of Schools, while admitting that much progress has been made in the use of museums in education, calls for a still greater use of them. The desirability of museums and educational institutions to cooperate to a greater extent is emphasized, as is the need for museums to cooperate more. The report says the "happy camaraderie among professionals needs to become a much more flexible and extensive exchange of exhibitions and professional services in many fields"

Clearing the Air from Geneva

from a Correspondent

THE second World Administrative Radio Conference for Space Telecommunications, organized by the International Telecommunication Union, was held in Geneva from June 7 to July 17, 1971, and was attended by some 650 delegates from 100 nations. The decisions taken at the first such conference in 1963 have stood the test of time, but developments in space technology and radio astronomy have made new international agreements necessary to cater for the needs of the next decade.

The demand for radio frequencies shows no signs of abating, and as there is a limit to the extent of the radio spectrum which can be exploited the same frequencies should be used by more than one service whenever this is technically feasible. Much of the discussion, and most of the difficulties in reaching agreements, were concerned with problem sharing. As technology advances some of the increased demands can also be met by using frequencies higher than 40 GHz, the previous upper limit of the allocated bands, and allocations have now been made up to 275 GHz.

There has been a remarkable expansion in satellite communications since 1963, and the next decade is expected to see the implementation of satellite services for aeronautical and maritime navigation and communication, for direct broadcasting to the public (at least on a communal basis), for the study of the Earth's resources, and for monitoring the environment. It is also becoming clear that recent discoveries by radio astronomers are providing new insight into the fundamental physics of matter, and that greater protection from interference in several frequency bands is necessary for this important branch of science to develop.

Most of the work of the conference was organized in three committees. The first was concerned with the administrative procedures by which international negotiations may take place as services are introduced or expanded. It has been necessary to revise the procedures laid down in 1963, particularly as the greater emphasis on frequency-sharing calls for clear and more comprehensive procedures by which different countries may coordinate their activities. An interesting departure from custom is that there is now provision for the adoption of new technical criteria on frequency sharing when these are recommended by the International Radio Consultative Committee (CCIR, an advisory committee of the ITU) without waiting for another administrative conference. Countries will have the option of retaining the existing criteria or adopting the revised criteria when these become available.

The second committee was concerned with establishing the technical criteria such as limits of radiated power and power flux densities which are considered necessary to enable services to share frequencies without mutual interference. This committee also provided the technical data needed first for calculating the coordinating distances within which consultation with other countries need take place, and then for calculating the likelihood of interference, and the necessary separation distances, taking into account the actual radio links in operation or planned. Agreement on comprehensive technical standards, in the available time, was made possible only by the thorough preparatory work carried out in February 1971 by a special joint meeting of relevant Study Groups of the CCIR in Geneva, and before that by a CCIR working party in Nice in December 1970, which assembled the latest data on radio propagation necessary to enable the more general studies to proceed.

Since 1963 it has become clear that the future development

of space communications is vitally dependent on the exploitation of geostationary satellites, which remain fixed in relation to the surface of the Earth. The technical committee therefore gave considerable attention to the need for the efficient use of the geostationary satellite orbit, and the criteria which must be adopted to avoid interference between links using different satellites in this orbit, and between these links and terrestrial services.

Although the preparation of administrative procedures and technical criteria is fundamental to the development of communications, it is the frequency allocations themselves which will excite the widest interest. The revision of the frequency allocation tables was the task of the third committee, and some difficult problems were encountered. Although space services can be operated over a wide range of frequencies, certain parts of the spectrum have marked advantages and are in heavy demand. One such range, for example, is in the region of 2-3 GHz; wide bandwidths are not available at much lower frequencies, and propagation at much higher frequencies can be seriously affected by adverse meteorological conditions. This desirable part of the spectrum, which is already used extensively for terrestrial services, is also in demand for Earth exploration satellites, for broadcasting satellites, and for increased bandwidths in space research. An acceptable compromise was finally achieved, by which it should be possible for these services to share certain bands, provided that there are only a few Earth stations for space research and Earth exploration, that the power flux density from broadcasting satellites is kept below an agreed limit, and that full consultation takes place before any new system is established. Similar problems in other parts of the spectrum were treated in similar ways.

The final agreements on frequency allocations are inevitably the result of compromise, and operators of most services will be disappointed that not all their hopes were realized. It has not been possible to make new primary allocations for space research, except in the newly-allocated part of the spectrum above 40 GHz, but several special provisions have been made which will, it is hoped, enable the necessary arrangements for specific operations to be made by negotiation. The basis of this philosophy is that successful operation of a few Earth stations in the space research service, even though a high degree of protection is required, need not inhibit the use of the same frequencies for other services in other parts of the world.

Radio astronomy has received improved status in several bands, and has obtained a new exclusive high frequency band near 22 MHz and new bands above 40 GHz. There is increased recognition of the importance of bands needed for observing natural line emissions. On the other hand, the expected increase in transmissions from satellites, for all purposes, constitutes a threat to radio astronomy which cannot be avoided by locating observatories in remote areas. As an example, there is now an allocation for broadcasting satellites in a band adjacent to the exclusive radio astronomy band at 2,690-2,700 MHz. Careful planning and much goodwill is needed if radio astronomers are to be able to use this band without danger of serious interference.

The new regulations will come into force, formally, on January 1, 1973. But as they reflect the established policies of most countries, many of the provisions will already have been adopted for practical purposes, and others will no doubt be followed well in advance of the formal date of implementation.

NEW WORLD

NRC Committee Knocks Anti-knock

by our Washington Correspondent

A STUDY of the health hazards associated with airborne lead, conducted by the National Research Council*, achieves the remarkable feat of providing sound biological reasons why lead should be banned from use in petroleum as soon as possible, while at the same time cutting the ground from under the feet of the most vociferous critics of the automobile industry. The chief conclusion of the study is that while airborne lead does not provide a serious threat to the health of the average city dweller—even in Los Angeles—young children and some groups of workers may get enough lead into their bodies from car exhausts to produce biochemical damage and, in severe cases, even clinical signs of lead poisoning. The study, which was conducted under contract to the Environmental Protection Agency by the Panel on Lead of the Committee on the Biologic Effects of Atmospheric Pollutants (a special committee of the National Research Council), will be scrutinized carefully by the EPA, for it is planning to introduce regulations controlling lead in petroleum in December this year.

Based on an extensive survey of the literature on lead pollution, the study comes up with the finding that the concentration of lead in the largest cities in the United States is twenty times greater than over sparsely populated rural areas, and more than 2,000 times greater than in the air over the centre of the Pacific Ocean. There is "little doubt as to whether man has substantially contaminated some parts of his environment with lead", the panel concludes, and "it is also clear that one major source of general environmental contamination is the combustion and dispersal of lead alkyl compounds used as automotive fuel additives".

Before this conclusion can be used as a stick with which to beat the car manufacturers, however, the panel points out that the average lead content in the air over major American cities has not changed greatly for the past fifteen years, and even if cars carry on discharging lead into the atmosphere, there will be little change in the magnitude of the effect on biological systems "for some years to come". The panel also concludes that much the most important

source of lead in the blood comes from food and drink. In any case, the average city dweller in the United States receives insufficient doses of lead from both the air he breathes and the food he eats to pose a threat to his health.

Although the panel offers no suggestion on the level of lead in the blood that can be considered safe, running through the report is the assumption that blood lead levels below 40 μg per 100 g are not biologically harmful because it is only above that level that synthesis of the haem part of the haemoglobin molecule is affected, and the first clinical symptoms of lead poisoning (which are usually mild anaemia, headaches and generalized muscle aches) are not noted until blood lead levels exceed about 80 μg per 100 g of whole blood. In contrast, the panel report cites studies that show that the average person living and working in the city of Los Angeles—where the concentration in the atmosphere is twice as high as most other cities—will have a blood lead content of less than 25 μg per 100 g of whole blood, and inhabitants of other cities are likely to have about 15 μg of lead in every 100 g of blood.

On the other hand, however, the panel puts children who live in large cities and some groups of workers, such as policemen on traffic duty and parking lot attendants, at a high risk from airborne lead. The latter group is more or less continually exposed to high concentrations of airborne lead, which may result in blood lead concentrations greater than 40 μg per 100 g of blood, but the panel also points out that "even within this small population, this level of blood lead concentration is probably attained by only a relatively small proportion of those exposed. To reach the very high level of blood concentration compatible with clinical lead poisoning would probably require in these special groups a five-fold increase beyond current levels in lead assimilation for a long period".

But the exposure of city children to airborne lead and also to lead paint and other sources is a much more intractable problem. For one thing, recent studies have shown that many city children have blood lead concentrations of 40–60 μg per 100 g. Leaving aside the ingestion of lead paint, which is a major problem in the poorer areas of large American cities, the panel suggests that children living in large cities

are exposed to a high risk of lead poisoning through dust and grit in the streets. Such dust often contains more than 2,000 μg per g of lead, and if a child manages to eat only about 40 mg of dust each day, his blood lead content could be taken above the 40 μg limit. Or, more drastically, if he ate 0.4 g a day, he would show symptoms of clinical lead poisoning, possible even resulting in damage to the central nervous system. This, says the panel, "is an undesirable level of exposure". Unfortunately, but not surprisingly, nobody is clear about how much dust and grit is swallowed or inhaled by children in large cities because no such studies have yet been reported. That is a social study which the panel suggests could well be carried out.

Asked last week whether these findings of blood lead concentrations call for a ban on lead in petroleum, Dr Paul B. Hammond, the panel's chairman, said "on strict biological grounds, I would say that a case could be made out for banning lead from gasoline, but I could also make out a case, on strict biological grounds, for banning the automobile as a lethal weapon". Dr Hammond pointed out that the panel can make a biological judgment, but it is now up to somebody else to make an economic judgment on whether or

Feasibility Study

THE National Academy of Sciences has accepted a contract from the Environmental Protection Agency to determine whether the automobile industry is capable of manufacturing an engine that will meet the standards set by the Clean Air Act. Manufacturers must, by 1975, produce automobiles which emit 90 per cent less carbon monoxide than the engines produced in 1970, and they have until 1976 to reduce emissions of nitrogen oxides by 90 per cent. A committee, under the chairmanship of E. L. Ginzton, chairman of Varian Associates, California, will determine whether it is technologically feasible for the industry to design, mass produce and service such an engine. Reports will be sent to the EPA and to Congress in January and July of each year.

**Airborne Lead in Perspective*, to be published by the National Academy of Sciences, Printing and Publishing Office, 2101 Constitution Avenue, Washington DC 20418.

not lead alkyls should be banned from petroleum. Ironically, it may be the manufacturers themselves who are forced to conclude that they can no longer afford to have their cars run on leaded petroleum, whatever recommendations the EPA comes up with in December. The automobile manufacturers must by 1975 meet strict regulations on the amount of unburnt gases and nitrogen oxides emitted from automobile exhausts, and one method which at present looks promising is to attach a catalyst to the exhaust system to reduce nitric oxide and to oxidize carbon monoxide. Lead, however, poisons the catalysts.

Apart from its findings on the levels of lead in the blood of city dwellers, the panel also pinpointed areas where more research is needed. For one thing, the panel points out that monitoring of both city air and food is totally inadequate to determine either the source of lead which eventually finds its way into human blood, or to provide warnings of potential risk. But the chief areas where more extensive research is called for lie in the biological sciences themselves. For example, the panel says that studies are required to determine whether the body burden of lead increases with age, and the assumption that the non-diffusible fraction is tightly bound in bone, and therefore biologically inert, also requires further detailed examination.

A more important recommendation, and one which throws into relief the panel's tacit assumption that blood lead levels below 40 g are safe, is the suggestion that studies on the biological effects of low levels of exposure to lead are urgently required. "The subtle effects on behaviour of low lead exposure of long duration without prior exposure may be manifest in two types of disorders: the dulling of mentation and chronic hyperkinesis", the panel points out. "No information is available regarding the possibility of cause and effect in what may be an extremely important problem."

TECHNOLOGY ASSESSMENT

Daddario's Kite Flies Yet

by our Washington Correspondent

THE Office of Technology Assessment, like an old soldier, refuses to die. A proposal to set up within the legislative branch of the Federal Government an agency designed to assess the likely consequences of technological projects has been kicking around in the House of Representatives Subcommittee on Research and Development for the past four years, and its ancestry can be traced back a good deal further than that. But, just as the idea seemed

finally to have been laid to rest, it has appeared again with enough vigour and support to take it through the rest of the congressional mill and probably even into the statue books. A bill to set up the office was introduced into the House by Mr John Davis, chairman of the Subcommittee on Research and Development and the rest of his committee members a few days before members of Congress dispersed for their Summer vacations. It was referred to Mr Davis' committee, and has since been sent back to Congress without amendment and with a report explaining why it would be "shortsighted in the extreme, if not disastrous" for Congress to turn the idea down.

The bill, which is in most respects identical with a bill introduced by Emilio Q. Daddario, Mr Davis's predecessor as chairman of the research and development subcommittee, would establish an Office of Technology Assessment charged with appraising the impacts of the applications of technology and with helping Congress to determine the relative priorities of the projects before it.

The chief reason for the bill, and without doubt its strongest asset on Capitol Hill, is that the Administration at present has all the resources at its disposal for making decisions on technological matters and Congress is ill-equipped to challenge them. To help redress the balance, the proposed Office of Technology Assessment (OTA) will provide information to congressional committees and serve as an early warning system for likely undesirable social effects of legislation involving technology. All these functions would be performed at a cost of \$5 million for the office's first year of operation.

Jobs specifically earmarked for the OTA in the bill are to identify existing or probable impacts of technology, where possible to establish cause and effect relationships between technological advances and social or financial consequences, to determine alternative programmes to achieve specific goals, to identify areas where more research is needed to support the assessment, and to furnish the results of these labours to the appropriate legislative body. The OTA will have the Library of Congress to help carry out its tasks, and the National Science Foundation to co-operate on the development of techniques of technology assessment, and to award grants and contracts for research needed by the OTA.

A panel consisting of eleven members would be responsible for the policy of the OTA and an operational unit, headed by a director, would be charged with the task of carrying it out. Two Senators, two members of the House of Representatives, the Comptroller General, the director of the Congressional

Research Service of the Library of Congress, the director of the OTA itself, and "four especially qualified members from the general public" appointed by the President with the Senate's approval would together provide the personnel for the Technology Assessment Board. The director would be appointed for a six-year term and the public members of the board would serve for four years each, with the possibility of being reappointed once. Assessments could be instigated by the board or the director, but the chief users of the office's expertise are expected to be congressional committees whose chairmen could ask for specific studies to be carried out. Like the FBI, the OTA would be expected to furnish a report without coming to any conclusions.

Although the way in which the OTA will in theory carry out its business, and the rationale that underpins the attempt to set it up, is clear, thirteen days of hearings and four years of rumination by the Subcommittee on Research and Development have failed to provide a convincing description of how it will work in practice. For one thing, if the OTA is to be used to check on a particular action of the Executive, it will have to move with sufficient speed to produce a report that is not a post mortem, and for another, independent members of the public who are both equipped and willing to serve on such a body are rare indeed. The new bill has at least alleviated the problem of finding staff for the Technology Assessment Board to the extent that it has cut the number of public members down from seven in the original proposal to six and finally to four in Mr Davis's new bill.

If the bill survives intact the congressional mill—as indeed it stands every chance of doing, for Congress is not noted for turning down measures that are designed to strengthen its hand against the Executive—the Office of Technology Assessment would be essentially Mr Daddario's baby, for it was he who introduced the legislation leading up to the new bill, and it was under his chairmanship that the subcommittee conducted its hearings. Although the need for Congress to be kept informed of the likely consequences of new technology was agreed by all the witnesses before the committee, few aspects of the proposal remained uncriticized. Dr William D. McElroy, Director of the National Science Foundation, for example, told the subcommittee that the office would lack credibility and that it may better serve to coordinate the work of agencies that are already engaged in technology assessment. Those are criticisms that the new legislation has clearly failed to answer.

MARINE POLLUTION

No More Dumping

by our Washington Correspondent

GONE are the days when politicians would complain that there are no votes in sewage, and that there is no political capital to be gained from supporting legislation to clean up the rivers and oceans. With the present public concern about the deterioration of the natural environment, sewage has become a live political issue, and one on which most politicians have something to say. Such was the case last week when the House of Representatives passed, by a vote of 304 to 3, a bill designed to regulate the dumping of waste material in the tidal waters around the United States, and to prohibit entirely the dumping of some particularly toxic material.

In many respects, the bill embodies in legislation the conclusions and recommendations of the Council on Environmental Quality, whose report *Ocean Dumping: A National Priority*, was sent by President Nixon to Congress in October last year. But the bill has already met with opposition from the Bureau of the Budget for extending the council's recommendations to include the designation of marine sanctuaries around the United States and for requesting a further \$2 million for research into the long-term effects of marine pollution.

Chief among the provisions of the bill are a total ban on the dumping of chemical and biological weapons and highly radioactive waste materials into the oceans, and a ban on the dumping of other material unless a permit has been issued by the Environmental Protection Agency or the Secretary of the Army. Disposal from outfalls is, however, not covered by the bill because it is already regulated by the Federal Water Pollution Control Act. Permits will be granted by the EPA, or the Secretary of the Army in the case of dredged or fill material, for transportation from the United States of the material to be dumped.

An applicant for a permit must convince the EPA that the dumping will not degrade the environment or present a danger to public health and he may, if necessary, be required to defend his application before a public hearing. If he decides not to bother with the bureaucracy involved in getting a dumping permit, he will be liable to a fine of \$50,000 for illegal dumping, and to help ensure that he is discovered in his crime, the bill proposes that members of the public be rewarded by up to \$2,500 for providing information which brings the offender to book.

As for research and development on the ecological consequences of marine dumping, the bill provides \$2 million

to the National Oceanographic and Atmospheric Administration (NOAA) to engage in both long-term and short-term studies. The Committee on Merchant Marine and Fisheries added this clause to the Administration's original proposals because it believes that an early warning system is needed to identify, for example, pesticide residues whose presence is likely to constitute an ecological hazard. Too often, such damage is noticed only when it has become irreversible. The bill also sensibly calls for wide-scale monitoring of the purity of the oceans. The proposal to give more government money to marine research has, however, already fallen foul of the Office of Management and Budget, for Donald Rice, an assistant director of OMB, wrote to Mr Thomas M. Pelly, chairman of the Committee on Merchant Marine and Fisheries, to complain that this section of the act is "unnecessary and undesirable". With curious logic, Mr Rice explained that since the EPA and NOAA are already spending more than \$2 million between them on oceanographic research, giving them an extra \$2 million may restrict their activities.

An even more curious aspect of the legislation dealing with research is the function envisaged for the National Science Foundation. In the original bill, the NSF was earmarked to receive an extra \$1 million along with NOAA, but during the debate, Mr Lennon, from North Carolina, deleted all reference to the NSF and instead substituted the Secretary of State for Commerce. Mr Lennon's amendments were taken immediately after a roll call, and in the confusion, or perhaps in the absence of interest from his colleagues, they were accepted without discussion or division. Mr Lennon later explained, however, that he had decided to cut the NSF out of the bill because NOAA is better equipped to manage the research programme, and by vesting the responsibility in the hands of a single agency, duplication of effort can be cut to a minimum.

The chief fight with the Administration over the bill is likely, however, to involve the section dealing with the designation of marine sanctuaries in which all sorts of activities would be prohibited in an effort to preserve the natural beauty or the wildlife of the sanctuary. NOAA has been charged with the task of deciding which areas of coastal water should be so designated, and the Secretary of State for Commerce will be given the authority to enforce whatever regulations are laid down to protect the sanctuary. Sanctuaries must be designated within two years after the bill is signed by the President, and their siting will be subject to public hearings.

The Administration's chief grumble is that this section of the bill is likely to cost up to \$10 million a year for the first three years, mostly for the acquisition of land. But the Administration is also chary of infringing the rights of states whose shoreline borders on a proposed marine sanctuary. A move to delete this section of the bill in the House of Representatives was, however, defeated by a vote of 20 to 33, lack of a quorum notwithstanding, and a motion seeking to prohibit oil prospecting in areas being considered for designation as sanctuaries was turned down with an even smaller vote—10 to 42. But the days of indiscriminate dumping seem over.

Space Collaboration

THE successful meeting of scientists from the USSR Academy of Sciences and from the National Aeronautics and Space Administration (NASA) at Houston in June is to be followed by another meeting between the parties in Moscow in November. The aim of the series of meetings is to discuss the technical requirements for a possible link-up in space between an Apollo-type spacecraft and a Salyut-type orbital scientific laboratory. Even further in the future a link-up between a spacecraft of the Soyuz-type and a Skylab orbiting station might be considered.

The problem facing the scientists is to agree on compatible systems of rendezvous and docking, radio and optical signals, communications, life support and crew transfer. After the Houston meeting it was agreed that the technical feasibility of accomplishing such a docking exists in principle and that further studies would be advantageous.

The current plan is to initiate the Skylab project at the end of the Apollo programme and to launch the first such Earth orbiting workshop in May 1973. Three different groups of astronauts will stay on board for up to twenty-eight days on the first mission and for up to fifty-six days on the second and third missions planned for the end of 1973.

A Salyut spacecraft is already in Earth orbit, and the first stage of the proposed collaboration will ensure that the best developed facets of the United States and USSR space programmes will be combined.

NEWS AND VIEWS

Haemoglobin Synthesized in *Xenopus* Eggs

THE rumours, which have been circulating for the past several months, to the effect that Gurdon and his colleagues at Oxford have observed the translation of mammalian messenger RNAs which were injected into frog eggs and oocytes, were well founded. For on page 177 of this issue of *Nature*, Gurdon, Lane, Woodland and Marbaix describe in detail how to use *Xenopus* eggs and oocytes as an extremely sensitive assay system for amounts of messenger RNA as small as 1 ng. That is not all; with their oocyte system it is now feasible to study the control of the translation of messenger RNAs in living eukaryotic cells. This development, coinciding as it does with the progress several groups have made towards the development of efficient cell-free systems which support the translation of eukaryotic messengers, could scarcely have been better timed. With these two approaches at their disposal, and with the wealth of information about bacterial protein synthesis at hand, biochemists should, with luck, now be able to elucidate the minutiae of protein synthesis in eukaryotes.

9S RNA from reticulocytes is, of course, the most readily available mammalian messenger and the results of initial experiments by Gurdon and his colleagues, which are to be published shortly in the *Journal of Molecular Biology*, proved that oocytes, taken from *Xenopus laevis* induced to ovulate by hormone treatment, synthesize haemoglobin when they are injected with this RNA and haemin. Greatly encouraged, Gurdon's group then set about characterizing more precisely this system and, what is perhaps even more important, proving that the haemoglobin messenger is not a special case and that other foreign messengers can be translated in *Xenopus* oocytes.

Their first problem was discovering the best way to introduce labelled amino-acids into eggs and oocytes. It turned out that injected ^3H -histidine leaks out of oocytes far more rapidly than out of eggs; conversely, oocytes take up this label from the medium more rapidly than do eggs. Deciding to label eggs by injection and oocytes by incubation in labelled medium, they followed the rate of synthesis of haemoglobin after the injection of rabbit 9S RNA and compared it with the rate of translation of endogenous messengers. After a lag phase of some 10 to 15 minutes, presumably the time taken for the injected messenger to become associated with the cells' translation apparatus, the synthesis of haemoglobin and the translation of endogenous messenger continues at a constant relative rate for more than 20 hours. Furthermore, experiments with puromycin indicate that both endogenous protein synthesis and haemoglobin synthesis are equally susceptible to inhibition. Plainly there is nothing atypical about the way in which the haemoglobin messenger is translated and the haemoglobin messengers are no less stable in oocytes and eggs than their endogenous counterparts.

The next question to answer was how efficiently do oocytes and eggs translate the rabbit haemoglobin messengers? By measuring the size and specific activity of the pool of histidine in these cells and the amount of this amino-acid incorporated into haemoglobin during given times after the injection of known amounts of messenger

preparations, Gurdon and his associates have made an estimate of the number of globin chains made per hour per molecule of messenger. Such estimates are inevitably only approximate for they depend on a variety of unproven assumptions. For example, the size of the pool of histidine in the cells which is drawn upon for protein synthesis might well be significantly less than the total amount of extractable free histidine, which was what Gurdon *et al.* measured. And factors such as the leakage of messenger from injected cells and the failure of some RNA molecules to act as messengers add further elements of uncertainty. Nevertheless the estimates provide a fairly reliable indication of the comparative efficiency of translation of haemoglobin messengers in different systems. It emerges that oocytes are almost as efficient at translating this messenger as are reticulocytes; at 20° C each molecule is translated about 80 times an hour in reticulocytes, 24 times an hour in the best oocyte preparations and a mere 0.1 times an hour in a cell free system, to judge from the published data of Lingrel.

But is the translation of haemoglobin messenger in eggs and oocytes a special case? Obviously if it is the system has only limited potential but, on the other hand, if *Xenopus* oocytes and eggs will support the translation of all manner of foreign messenger RNAs as efficiently as they support haemoglobin synthesis there are countless ways in which this system might be exploited. Unfortunately with so limited a selection of natural messenger RNAs currently available in anything like a pure form, Gurdon's group can only provide a partial answer to this crucial question but their answer is most encouraging. Of the three messengers which they tested a 9–11S RNA from mouse myeloma cells and the synthetic messenger AUGpolyU seem to be translated but phage f2 RNA is not. Oocytes receiving the myeloma messenger incorporate ^3H -serine and ^3H -threonine into protein which is precipitated by antisera against the myeloma protein and which co-chromatographs on 'Sephadex' with cold carrier myeloma protein. Synthetic AUGpolyU promotes the polymerization of phenylalanine and concomitantly endogenous protein synthesis is severely restricted. In short, more than one natural messenger and a synthetic messenger is translated.

As Gurdon *et al.* point out, this means that oocytes can be used as an assay system for the putative messenger activity of RNA preparations from eukaryotic sources. If injected RNAs either stimulate amino-acid incorporation by these cells or compete with the translation of haemoglobin messenger RNA they will probably be found to contain messengers. And as an example of the sensitivity of this system they show that as little as 2.5 ng of RNA from mouse 3T6 cell polysomes almost halves the amount of haemoglobin made by oocytes injected with saturating amounts of haemoglobin messenger. By contrast 30 ng of ribosomal RNA has no effect on the amount of haemoglobin made. Indeed by increasing the amount and number of radioactive amino-acids in the cells, Gurdon's group calculate that after incubations of about 20 hours it may be possible to detect as little as 1 pg of haemoglobin messenger.

GERONTOLOGY

Living Longer

from a Correspondent

AT the international forum on the control of human ageing, held from September 2-4 at the Gottlieb Duttweiler Institute (The Green Meadow Foundation) in Switzerland, two different points of view became apparent—there were those participants who stated that research in life prolongation must continue and those who were appalled by the social and economic aftermath of such added years.

According to Professor F. Verzar (Institutes für Experimentelle Gerontologie, Basel), this century will be the century of the aged and progress will be made in social gerontology, medical geriatrics and experimental gerontology. A move to the right of the human taximeter clock giving a 10-20 per cent increase in life span may come and batteries of tests to measure ageing rate in the short term must be used. Calorie restriction remains the simplest method of prolonging life and if any active agent such as an antioxidant is used it should be capable of being introduced as late in life as possible to prolong active vigorous life without increasing dependent old age.

The search is now on for a substance which will turn off the process of ageing. Professor B. L. Strehler (University of California) suggested that if the human thermostat could be reprogrammed to 2° C or less life would be increased by 25 per cent.

According to Dr L. Robert (CNRS, Laboratoire de Biochimie du Tissu Conjonctif) immunological manifestations of age related disease are probably themselves part of a general gene-related, differentiation-linked ageing mechanism. Orgel's (*Proc. US Nat. Acad. Sci.*, **49**, 517; 1963) theory of the inaccuracy of the protein synthesizing machinery of cells was supported by work with a strain of *Neurospora* (Dr C. M. Lewis, West London Hospital).

Professor I. Sobel (Florida State University) spoke on the employment problem of the over forty-fives. Older workers are already on average unemployed longer. Adding fifteen years to the life span would lead to a period of decline in earnings lasting for twenty-five to thirty years with serious consequential psychological upsets. Each 5-year increase in life span would raise the labour force by about 5 per cent.

Professor W. F. Anderson (University of Glasgow) considered that prolongation of active human life without massive retraining programmes for the older worker and without encouragement to take up part-time employment associated with adequate medical and social resources for the existing elderly would be disastrous.

The pharmacological control of ageing was reviewed by Dr C. G. Kormendy (Bristol Laboratories, Syracuse). He suggested that the most promising substances should be subjected to laboratory studies. Most agents still seem to be too toxic.

Dr M. Goldsmith (Science Policy Foundation, London) stressed that vast socioeconomic implications must be considered before institutions of gerontology are established. Fortunately, gerontologists are unlikely to contribute to demographic pressure before the year 2000 and antioxidants, perhaps the least expensive agents, would have no significant effect until the early decades of the twenty-first century (Dr R. W. Prehoda, Toluca Lake Station, California). Dr R. D. Clarke (Prudential Insurance Company, London) thought that, by 1990, therapies based on two or more ageing theories may extend the

life span by 35 per cent. Any modest increase in contraception, abortion or sterilization would have greater impact on demographic pressures in the next thirty to forty years than the probable advances in gerontology.

In discussion, pleas were made for controlled studies now of the effect of certain endocrine substances, for example oestrogens in women, regarding their influence on the life pattern, and Dr J. Huet (International Center of Social Gerontology, Paris) made a forceful argument for an international institute of gerontology under Professor Verzar's administration.

The participants, although differing in the benefits of and means of prolongation of life, were unanimous in the need to plan now for larger numbers of old people. Multidisciplinary action should be taken by scientists, sociologists and physicians.

Drug Resistance in Cancer Chemotherapy

THE problem of the development of resistance to chemotherapy is one of the major difficulties in the path of successful treatment of disseminated cancer. A study of the mechanisms of resistance in man is extremely difficult for most cancers because remissions are uncommon and multiple biopsies of cancer material are not usually possible. A study of resistance to drugs in acute leukaemia, however, is feasible because multiple complete remissions are common and the blood and bone marrow can provide malignant cells at several stages during the development of the disease.

Drug resistance may develop because the agent is not taken up by the malignant cell or because enzymes are produced which catalyse its destruction either in the malignant cell itself or by normal tissues such as the liver. New metabolic pathways may be used to circumvent the metabolic block of the agent used. These types of resistance are likely to be specific for a single group of chemotherapeutic agents with similar modes of action. Another possible mechanism of resistance is less specific and depends on an alteration in the proliferation characteristics of the cancer cell. Such changes could result in an alteration in the effect of all drugs acting on cancer cells at the time of DNA synthesis which includes most anti-cancer chemotherapeutic agents. Resistance to a large number of drugs could therefore develop non-specifically. There is growing evidence that all these mechanisms may be operative in man.

One example of resistance to a drug being modified by metabolic alterations within the cancer cell is provided by the treatment of acute lymphoblastic

leukaemia using L-asparaginase. This agent is thought to act, at least in part, by depriving the malignant cells of the amino-acid L-asparagine. Resistance to L-asparaginase which develops during or following treatment has been associated with increased levels of asparagine synthetase activity within the leukaemic cell (S. M. Haskell *et al.*, *Cancer Res.*, **29**, 974; 1969).

The response of acute leukaemia in man to treatment with the antimetabolite cytosine arabinoside has also shown a correlation with the rate of uptake of the drug *in vitro* and its subsequent activation by phosphorylation (M. Y. Chu and G. A. Fisher, *Biochem. Pharmacol.*, **14**, 333; 1965). Stewart and Burke report in next Wednesday's *Nature New Biology* that leukaemic cells from patients who respond to treatment contain six times less cytosine deaminase than is present in cells of those who do not respond. The concentration of this enzyme, which inactivates cytosine arabinoside, increases following a course of therapy and this is correlated with increased resistance to therapy.

Tests *in vitro* in man for predicting response to the initial treatment using different drugs are unfortunately unreliable—the tests pay no attention to an agent's absorption, detoxication, excretion, distribution and duration in the body. The mechanisms whereby cells can repair or circumvent damage by the agent may be very different *in vivo*. Nevertheless, in acute leukaemia it may be possible to predict the development of resistance, following one course of treatment, to a particular drug. This could well be important in the management of patients relapsing with this disease.

ENZYMES

Protease Prospects

from our Molecular Biology Correspondent

It seems to be a general rule of nature that animal proteases are manufactured in a harmless packaged form, as zymogens, and are activated, generally by the intervention of some other protease, when the organism has need of them. This process may have evolved in part as a device to prevent the onset of a lethal proteolytic activity on completion of biosynthesis, and, more interestingly, as a refined control mechanism, as reflected, for example, in the role of the enzyme cocoonase. This is a serine protease, produced by the silk moth for use as a chemical crowbar to break open its cocoon. It is synthesized as a zymogen, prococoonase, in cells at the mouth of the insect, is deposited on the surface, where it forms a solid crystalline crust, is activated, and then dissolved in a specially secreted potassium bicarbonate solution, with a pH corresponding to the activity optimum. The solution seeps into the cocoon, and dissolves out the sericin, a proteinaceous "silk glue", and leaves the moth free to pass into the outside world.

The latest in a series of articles from the two laboratories which have uncovered this scheme (Berger, Kafatos, Felsted and Law, *J. Biol. Chem.*, **246**, 4131; 1971) now describes the nature of the zymogen, and the manner of its activation. An extract of the secretory parts of the insect, at a point in its development some days before emergence, contains no cocoonase activity. The attempts first made at activation of the prococoonase in this solution with active cocoonase resulted in rapid loss of the activity of the latter. This, it transpired, was a consequence of attack by a tyrosinase present in the blood. When this enzyme was inhibited the cocoonase was seen to bring about gradual activation. Trypsin or subtilisin, on the other hand (but not chymotrypsin), caused very rapid liberation of the full cocoonase activity, and it was therefore surmised that free cocoonase can play only a secondary role in activation. One does not, however, have to search far for a source of activating protease activity: at an early stage of adult development the region between the adult and the pupal cuticle is full of a liquid, the moulting fluid, rich in proteases, which serves to digest the pupal cuticle. When the fluid has done its work it is reabsorbed, leaving apparently a residuum of proteases to look after the prococoonase.

Estimates of molecular weights by a number of methods showed that the molecular weight of the zymogen was about 32,000, some 8–10,000 more than

free cocoonase. Thus by contrast with trypsin, which it otherwise closely resembles, a large part—about one quarter—of the chain is lost on activation. Benger *et al.* point out that because the activation fragments of zymogens are in effect nothing more than covalently bound inhibitors, the structural requirements may be supposed to be much less stringent than for the active enzyme, and that large differences introduced in the course of evolution in these parts of the chain are not perhaps so very surprising.

Another new zymogen has been discovered by Harper, Bloch and Gross (*Biochemistry*, **10**, 3035; 1971). This is the precursor of the collagenase which the tail of the tadpole carries, as the means for its own annihilation when the moment of metamorphosis is at hand. Collagenases have been widely identified in animal tissue, and especially in diseased conditions, in which the elimination of collagen masses is desirable. The proenzyme was discovered through the observation that anti-collagenase antibodies were absorbed by

collagenase-free extracts of tadpole tail-fin tissue. The chromatographic fraction of the extract believed to contain the zymogen failed to generate collagenase activity on treatment with trypsin or chymotrypsin. When, however, the fraction was mixed with a medium in which living tail-fin tissue had been maintained for some days, and incubated at 37° C, activation ensued. The collagenase could be detected by its effect on the viscosity of the substrate, and by the appearance of tropocollagen fibrils with characteristic periodicities in the electron microscope. The collagenase produced under these controlled conditions was identified with the usual preparations by its inhibition by anti-collagenase antibodies. The collagenase has apparently the remarkably high molecular weight (assuming the reliability of the detergent-gel electrophoresis method) of 110,000, or so, and the zymogen seems to be slightly larger. The activating factor is easily inactivated by heat, and is evidently a specific protease, that appears only at the right moment.

Precursors of DNA Replication

THE biochemistry of DNA replication as opposed to DNA repair is still a great enigma and, according to Werner, so it will remain if those intent on elucidating it persist in assuming that DNA replicase uses the deoxynucleoside triphosphates as immediate precursors. For in next Wednesday's *Nature New Biology*, Werner describes experiments which lead him to the conclusion that the enzyme responsible for the semi-conservative replication of DNA in *Escherichia coli* polymerizes some activated form of the deoxynucleoside monophosphates.

Earlier this year, Werner reported in *Nature* (**230**, 570; 1971) that short pulses of ³H-thymidine are incorporated preferentially in small DNA chains, Okazaki pieces, whereas short pulses of ³H-thymine are preferentially incorporated into larger DNA chains. From this he argued that *E. coli* can differentiate between thymidine and thymine using the former for repair DNA synthesis and the latter for replication. His latest experiments are an attempt to identify the pathway of incorporation of ³H-thymine into DNA. When there is a dynamic equilibrium between the intracellular nucleotide pools—a steady-state condition—the pool of thymidine monophosphate is maximally labelled about 1 min after the cells are exposed to ³H-thymine. Maximum incorporation of the thymine into DNA and maximal labelling of the pools of thymidine diphosphate and triphosphate are attained after about 10 min. TMP is therefore not itself the

immediate precursor of DNA replication but either TDP or TTP might be.

When, however, the cells are fed ³H-thymine and simultaneously deoxyadenine or thymidine, which act as donors of deoxyribose, the pool of TMP is greatly increased and is maximally labelled within about 10 s and the maximal rate of incorporation of the thymine into DNA is reached within 20–30 s. The pools of TDP and TTP are, however, not maximally labelled until about 2.5 min. From these observations and measurements of the specific activities of the pools of TMP, TDP and TTP Werner concludes that although TMP is not itself an immediate precursor of DNA replication it is on the direct pathway to replication while TDP and TTP are not, and has to be converted to an activated form of TMP before being incorporated into DNA.

Because the size of the pool of activated TMP precursor is much smaller than the pool of TTP, and because DNA is replicated so very rapidly Werner suggests that simple diffusion and collision would be too slow to support the selection and alignment of the activated monophosphate precursors by the DNA replicase. He speculates therefore that some protein like that described by Alberts and Frey (*Nature*, **227**, 1313; 1970) may unwind the DNA duplex ahead of the replicase. The exposed single strands can then act as templates for the preselection and alignment of the precursors and all the DNA replicase has to do is link them together by phosphodiester bonds.

MITOCHONDRIAL DNA

Symmetric Transcription

from our Cell Biology Correspondent

THE transcription of double helical DNA by DNA dependent RNA polymerase is generally assumed to be an asymmetric process with only one of the two strands of the DNA, the coding strand, being transcribed into messenger or one of the stable species of RNA. In *Escherichia coli*, as Travers, Bautz and Burgess and their associates have shown, the ability of RNA polymerase to select and transcribe the coding strand depends on the presence of sigma factor, without which transcription is symmetric.

Mitochondria which seem to have evolved from symbiotic bacteria, not only contain small double stranded and closed circular DNA molecules but also their own RNA polymerase, which is distinct from that in the nucleus of eukaryotic cells. As Kuntzel and Schäfer reported recently (*Nature New Biology*, **231**, 265; 1971), the RNA polymerase in the mitochondria of *Neurospora crassa* is a much simpler and smaller molecule than *E. coli* RNA polymerase; it comprises a single polypeptide chain with a molecular weight of about 64,000. On reflexion therefore, Aloni and Attardi's report (*Proc. US Nat. Acad. Sci.*, **68**, 1751; 1971) that both the strands of the mitochondrial DNA of HeLa cells are transcribed *in vitro* is perhaps not all that surprising. Symmetric transcription may perhaps be the evolutionary price mitochondria have had to pay for their simple genome and its RNA polymerase.

The two strands of HeLa cell mitochondrial DNA can be separated by appropriate centrifugation, and Attardi and his colleagues have developed methods for isolating mitochondrial RNA made *in vivo* after pulse labelling with uridine. Determining whether transcription is symmetric or asymmetric can therefore be determined by comparatively straightforward DNA-RNA hybridization experiments. Aloni and Attardi find that RNA made in the mitochondria during short (1-5 min) pulses hybridizes equally to the heavy (H) and light (L) strands, but RNA made during longer pulses hybridizes preferentially with the H strand. They have already obtained evidence which indicates that the entire H strand is transcribed, and this together with the finding that RNA made during short pulses hybridizes equally with H and L strands, and the rapid sedimentation of the L strand RNA, suggests that like the H strand the L strand is extensively and perhaps completely transcribed. The L strand transcript, however, rapidly disappears from the mitochondria either because it is degraded or possibly because it is exported to the cell cytoplasm. Why the transcription of mitochondrial

DNA is an exception to the rule of asymmetric transcription remains to be seen.

Schäfer and Kuntzel and their colleagues (*Europ. J. Biochem.*, **21**, 478; 1971), having isolated the RNA polymerase of *N. crassa* mitochondria, have now devised a method for isolating intact the DNA from these mitochondria. They find it is a circular DNA some 25 μ m long, in other words some five times longer than the mitochondrial DNA of HeLa and other vertebrate cells; this leads them to speculate that the mitochondria in fungi may represent an early stage in the reductive evolution of bacterial endosymbionts which culminated in the mitochondria of vertebrates with their very small genomes. Using mitochondrial DNA from *N. crassa*, Schäfer *et al.* have investigated its transcription *in vitro* by *E. coli* RNA polymerase with sigma factor and conclude that there are about eight to nine specific binding sites for this mitochondrial genome. Some 50 per cent of the RNA transcribed *in vitro* by the bacterial enzyme seems from hybridization experiments to be ribosomal and transfer RNA. Among the remainder of the *in vitro* product are RNA strands which self anneal and may well be the product of symmetrical transcription of

regions of the genome other than those which specify the stable mitochondrial RNAs. As Schäfer and Kuntzel have themselves shown, however, the mitochondrial RNA polymerase is very different from the bacterial enzyme and it would be interesting to see what RNAs are transcribed *in vitro* by the mitochondrial enzyme.

STOMATA

Response to Humidity

from a Correspondent

TRANSPIRATION in plants is controlled by adjustments in the size of minute pores, the stomata, which perforate the surface of leaves. Each stoma consists of a slit bounded by two specialized epidermal cells, or guard cells, which determine its size. When the guard cells take up water and swell their shape alters in such a way that the pore opens and when they become less turgid it closes. Stomata are normally in an open or partly-open condition during the day and closed at night. As a result, diffusion of carbon dioxide into a leaf, which follows the same pathway as

Seismic Signals from Underground Explosions

IN next Monday's *Nature Physical Science*, A. Douglas, P. D. Marshall and D. J. Corbishley, from UKAEA, Blacknest, suggest reasons why some of the seismic signals from underground explosions are more complex than others. The basis of their idea is that the direct P waves recorded in a complex signal have passed through material with a lower average value of the quality factor, Q , and have been attenuated.

Most P signals from underground explosions are simple in character and usually comprise a single pulse—two to three cycles long—followed by a tail of lower amplitude arrivals which can be explained in terms of reflexions in the crust or upper mantle. But the less common complex signals have a tail with an amplitude that is similar to or even larger than the first arrival.

Douglas *et al.* point out that, according to their theory, the tails of complex signals should contain more high frequency energy than the direct P waves because high frequencies are selectively attenuated in materials of low Q ; complex signals from an underground explosion should therefore point to lower average explosion magnitudes than the simple signals because of the reduction of the amplitude of the first arrivals. To test the whole theory Douglas *et al.* have examined the P seismograms of two underground explosions—one fired

near Bukhara in the Soviet Union and the other (the nuclear explosion Longshot detonated on October 29, 1965) at Amchitka in the Aleutian Islands.

The vibrations from the Russian explosion were detected at Eskdalemuir in Scotland and at Gauribidanur in India, and the records from these two stations provide good examples of simple and complex signals from the same source. The record at Gauribidanur is complex and there is clear evidence of late arrivals with a higher dominant frequency than the earlier arrivals. And the magnitude of the explosion as measured from the Eskdalemuir records is higher than that indicated by the Gauribidanur data, suggesting that the direct P waves arriving at Gauribidanur had been attenuated.

Douglas *et al.* detail two examples of complex signals from the Longshot explosion (both recorded in British Columbia) from which the magnitude of the explosion was estimated to be 4.8 and 5.4; this is much lower than the average estimate of 6.2 from the data of the arrays operated by the United Kingdom Atomic Energy Authority which all detected a simple signal. They also find that the beginnings of the complex signals have a dominant frequency of ~ 0.8 Hz compared with a dominant tail frequency of ~ 1.4 Hz, again in accord with the theoretical predictions.

water vapour, is facilitated during periods of photosynthesis, and water loss is curtailed at night. But stomata also close sometimes during the day where the air is dry and excessive evaporation places the plant under water stress.

It is now well established that light causes stomata to open by lowering the carbon dioxide concentration in the vicinity of the guard cells as a result of photosynthesis. By this device increased consumption of carbon dioxide by the leaf leads to stimulation of absorption and vice versa. The response to changes in the humidity of the air has been less intensively investigated, but it has been generally accepted that it depends on alterations in the water content of the leaf as a whole. Lange, of the Botanical Institute, University of Würzburg, has dissociated himself from this view and in a recent article in *Planta* (100, 76; 1971) he and his colleagues put forward further evidence that guard cells respond directly to changes in the humidity gradient existing between the external air and sub-stomatal air space.

Lange *et al.* have devised an elegant technique whereby it is possible to observe microscopically the behaviour of stomata in isolated strips of leaf epidermis when subjected to rapid changes of ambient air of different moisture contents. Induction of opening and closing by alternate exposure of the outer surface of the epidermis to moist and dry air was repeated as many as fifteen times on the same stoma. When different areas of the same piece of epidermis were treated separately the stomata in each part responded independently according to the conditions to which they were subjected.

Lange *et al.* conclude that guard cells act as humidity sensors which respond directly to changes in the gradient of water potential between the air above the leaf and in the sub-stomatal air space. When this gradient increases the guard cells lose water and the pore closes and when it falls they take up water causing the pore to open. This mechanism causes a rapid adjustment in pore size in response to changes in evaporative conditions in the vicinity of each stoma irrespective of the water status of the leaf as a whole. Lange *et al.* assume that the level of carbon dioxide was unaffected by the treatments that were applied and that the changes in turgidity of the guard cells is attributable to a direct influence of the humidity gradient on absorption and loss of water by these cells. The possibility of an interaction between carbon dioxide concentration and water potential gradient in the overall control of stomatal response will no doubt be examined in the future extension of this work.

Satellite Lines in the Solar X-Ray Spectrum

IN next Monday's *Nature Physical Science*, J. H. Parkinson of the University of Leicester reports observations of satellite lines in the solar X-ray spectrum. These are lines which are observed close to the resonance transition of each helium-like ion, hence the name "satellite".

During the past few years the study of helium-like ion spectra and associated satellite lines has proved to be of considerable interest to solar physicists. In addition to the resonance line, a partially forbidden "intercombination" line is observed for each ion. A further strong line at slightly longer wavelength than the resonance and intercombination lines was shown by Gabriel and Jordan (*Nature*, 221, 947; 1969) to originate from a transition which was previously thought to be highly forbidden as a one quantum transition. These authors (*Mon. Not. Roy. Astron. Soc.*, 145, 241; 1969) then showed that the relative intensity of the intercombination and forbidden lines can depend on the electron density of the emitting plasma.

The satellite lines are caused by transitions in the stage of ionization lower than the helium-like ions, that is, in the lithium-like ions. The resonance line of the helium-like ion is a $1s^2-1s\ 2p$ transition. The satellite lines have a similar wavelength to this transition because they are transitions of the same type but in the presence of a further electron (that is, $1s^2\ n\ell-1s\ 2p\ n\ell$). Satellite lines have been observed in laboratory spectra for many years—Edlén and Tyrén first reported them in 1939, and proposed the forementioned origin.

As part of the investigation leading

to the identification of the helium-like ion forbidden transition, Gabriel and Jordan classified several multiplets of satellite lines, because it was at one time suspected that the strong solar line could have a similar origin. They proposed that the excited levels giving rise to the satellite lines are populated by the process of di-electronic recombination, hitherto not reported as a process important in laboratory plasmas, but thought to be important at the lower densities in the solar corona.

Several groups of workers (at the Aerospace Corporation, the Goddard Space Flight Center, the Lebedev Institute, and the US Naval Research Laboratory) have recently reported the presence of weak satellite lines in solar spectra, chiefly during flare activity. The new observations by Parkinson were obtained with improved spatial resolution by using a collimated crystal spectrometer, and refer to a stable phase of the active region. Parkinson's intensity data indicate that, as in the laboratory, the solar satellite lines are formed by the process of di-electronic recombination; this demonstrates directly the existence of this process in the solar corona. The relative intensities of the satellite lines and helium-like resonance lines are therefore dependent on the electron temperature in the emitting plasma. Parkinson has observed the intensities of the helium-like lines and satellite lines, so both the electron density and temperature in the emitting solar active region can be studied. And because each group of lines occurs over a limited wavelength region it should be possible to obtain reliable relative intensities.

Aspirin and Human Embryonic Cells in Culture

IN next Wednesday's *Nature New Biology*, Paine and Nagington report that sodium salicylate—a breakdown product of the common sodium acetylsalicylate or aspirin—inhibits the growth of some human embryonic cells in culture.

Tissue cultures from skin, lung, heart and kidney were prepared from 12, 14 and 18-week old human embryos. The cells were grown in the presence of various concentrations of sodium salicylate. Kidney cells from 12 week old embryos showed the highest sensitivity—2 mM (320 $\mu\text{g}/\text{ml}$) salicylate completely inhibited growth and 0.5 mM (80 $\mu\text{g}/\text{ml}$) almost reduced the growth rate by half. Lung cells were also affected, but less markedly. Heart cells were even less sensitive than kidney and lung cells; only 2 mM (320 $\mu\text{g}/\text{ml}$) salicylate showed any significant effect at all. Skin fibroblasts were not inhibited, even by 2 mM salicylate.

The mode by which salicylates produce this inhibition is unknown. Salicylates have a wide variety of pharmacological effects. There are indications of a correlation between salicylates taken during early pregnancy and foetal defects; but a causal connexion has not been established. Analysis of the foetal blood of 272 consecutive deliveries showed that about 10 per cent had salicylate concentrations of more than 10 $\mu\text{g}/\text{ml}$; the mean concentration in these infants was 33 $\mu\text{g}/\text{ml}$ (about 0.2 mM) (Palmisano and Cassady, *J. Amer. Med. Assoc.*, 209, 556; 1969).

Paine and Nagington quite properly point out that one cannot infer that the action of a drug in an intact animal or human is similar to the effect of the drug on cells in tissue culture. But the evidence they present is enough to warrant further investigations.

BIOENGINEERING

Aids for the Disabled

from a Correspondent

THAT the successful design of an aid for the disabled depends on detailed knowledge of the behaviour of the normal was clearly illustrated at the conference on human locomotor engineering held at the University of Sussex from September 7 to 10. The Institution of Mechanical Engineers had assembled speakers from many disciplines and discussion was both brisk and fruitful.

From a number of contributions dealing with the characterization of individual gait patterns in the various phases of the walking cycle, using electromyographic, cine-photographic or force-plate recording, it became clear that there is a need for more data about the functional anatomy of the limbs, and in particular about the exact positions of the lines of action of the various muscles relative to the centres of rotation at the relevant joints. Without this information it is difficult to calculate the force which any specific muscle exerts at a particular time or what the stresses are on the ligaments or articular facets of a joint. Quantitative descriptions of the time courses of several parameters in the gait pattern are required both for the design of the dynamics of artificial limbs and also in assessing the fitting of particular limbs to individual patients. Some of the walkway studies, such as that described by Dr M. Milner (Groote Schuur Hospital, Cape Town) and Dr T. Kasvand (National Research Council, Ottawa), now incorporate computer-scanning of the cine-film to derive the paths of markers attached to the subject over the axes of rotation at the various joints.

The recordable electrical activity of the muscles proves disappointing as a guide to the magnitude of the forces, but it does give useful timing information and an ingenious technique of display has been devised by Dr D. S. Grieve and Mr P. R. Cavanagh (Royal Free Hospital, London). Instantaneous values of the angles at adjacent joints, say, knee and ankle or knee and hip, are plotted against one another during the walking cycle and the resulting locus is marked off to indicate the period during which a specific muscle is active. The patterns generated in this way can be recognized by eye and may prove useful in recording the progress of clinical conditions.

Knowledge of the normal patterns of muscle activity also provides the basis for programmed electrical stimulation of muscles to restore a lost function. Dr E. M. Sedgwick and Mr S. A. G. Chandler (University of Southampton) have developed a neat trick of feeding back the amplitude of the M wave of the electromyograph in a servo to give

proportional control of the number of motor units stimulated. This method overcomes the difficulty that the range of intensities that lie between threshold and maximal stimulation is small and is comparable with the variation in the threshold itself.

In the design of artificial joints for implantation, there are many conflicting requirements. Dr P. S. Walker (The Hospital for Special Surgery, New York) concluded that the chief long term problem now is the reduction of wear at the surfaces of contact. New materials need to be tried. The properties of the adhesives at present in use also leave something to be desired, particularly where tensile stresses are involved.

In the field of simulations, Mr D. C. Witt and Mr J. I. Hall (University of Oxford) have made considerable progress with their automatically stabilized powered walking device. This device is about five feet tall and stands on small feet at the ends of telescopic legs a few inches apart. The leg lengths are adjusted by hydraulic actuators to give alternating support, stability being achieved by adjusting the time of dwell

on the appropriate side. They use a device modelled on the semicircular canals of the inner ear as an attitude sensor to control the switch-over. In a film, the "robot" was shown walking on the spot and it was not unbalanced when a block of wood was suddenly inserted under the descending foot. Symmetrical fore-and-aft motion of the legs has now been incorporated and the device is making its first steps in forward locomotion.

Professor J. M. Nightingale and Mr R. W. Todd (University of Southampton) described a robot hand which provides complex finely-adjusted movements of the digits to perform acts initiated by electromyographic signals from the operator (patient). The control signals that can be picked up from a patient and made available to control an external device are very severely limited in their information-carrying capacity. Adjustments of the digits to take account of the shape or fragility of the object to be grasped thus need to be delegated to an automaton which can take over the "unconscious" elements in the control task.

Measuring Epstein-Barr Virus Genomes

WHETHER or not Epstein-Barr (EB) virus has any role in the aetiology of Burkitt's lymphoma or nasopharyngeal carcinoma is still a matter of controversy and there seems little prospect of the issue being decided in the next several years. On the other hand, it is now generally accepted, chiefly as a result of the hybridization experiments done by Zur Hausen and his colleagues (*Nature*, **227**, 245; and **228**, 1056; 1970), that cell lines obtained from Burkitt lymphoma biopsies may contain detectable amounts of EB virus DNA even though these cells do not produce progeny EB virus particles. That is of course a familiar situation to tumour virologists who for the past several years have argued about precisely how many polyoma virus or Simian Virus 40 genomes are associated with the genome of a transformed cell. And now the same arguments are arising among those studying EB virus, for in next Wednesday's *Nature New Biology*, Nonyama and Pagano suggest that the so-called Raji line of Burkitt lymphoma cells may contain about sixty genome equivalents of EB virus DNA rather than about six equivalents, which was the estimate of Zur Hausen's group.

Zur Hausen's estimate was based on measurements of the extent to which EB virus DNA hybridizes with DNA from Raji cells and Nonyama and Pagano have measured the extent to which hot RNA, transcribed *in vitro* off EB virus DNA by *Escherichia coli*

RNA polymerase, will hybridize with Raji cell DNA. The advantage of this second technique is simply that the RNA can be made some 100-fold hotter than EB virus DNA. Using their high specific activity RNA, Nonyama and Pagano find some 700 genome equivalents of EB virus DNA in a line of Burkitt cells which produces progeny EB virus and some sixty-two genome equivalents of EB virus DNA associated with the mitotic chromosomal DNA of the non-producer Raji line. They also detect between forty-five and a hundred genome equivalents of this viral DNA in human leukaemic and normal leukocytes culture, cells which do not carry detectable amounts of EB virus antigens. By contrast the human HeLa cell does not contain detectable amounts of EB virus DNA, and Nonyama and Pagano believe that their data support the idea that infection by EB virus is required for the continuous growth in culture of human leukocytes.

What Sugawara, Mizuno and Osato have to say in next Wednesday's *Nature New Biology* should also interest anybody working with EB virus. By centrifugation through discontinuous gum acacia density gradients they have separated, by virtue of differences in buoyant density, EB virus carrier cells from cells actively supporting the replication of this virus. As the active replication of the virus in carrier cells is initiated and progresses so the buoyant density of the cells seems to decrease, and their separation becomes possible.

Reminiscences of Rutherford

from a Correspondent

At the thirteenth international congress of the history of science held in Moscow, August 18–24, one day (August 20) was devoted to a colloquium on the Rutherford centenary and 75 years of radioactive studies. The USSR Academy of Sciences extended a generous invitation to many of Lord Rutherford's former members of staff and students of the Cavendish Laboratory to attend this colloquium and to spend many days in the Soviet Union as guests of the academy. Academician Peter Kapitza (Institute for Physical Problems, Moscow) welcomed the rather small group of his old colleagues who had been able to accept the invitation; he had asked them to include in their addresses personal reminiscences of Lord Rutherford and the Cavendish Laboratory as it was in the 1920s and 1930s.

Professor Kapitza's acquaintance with Rutherford extended over more years than that of any other speaker; he had come to England in 1922 and remained as a member of the staff until his return to Russia in 1935. He spoke of the lessons he had learned from his Cambridge days and the great debt he owed to Rutherford who was not only an outstanding scientist but was the head and organizer of the most famous school of physics at that time. The genius of Rutherford lay in the speed with which he revealed the laws of nature and also in his remarkable talent for selecting young men with creative ability. Professor Kapitza recalled that only an accident had brought Rutherford to England: there were two candidates for the one 1851 Exhibition scholarship offered to New Zealand that year and Rutherford was placed second; fortunately for the world the winner decided not to leave New Zealand. Yet reading Rutherford's earliest works, which today are of no consequence and have sunk into oblivion, even at that time his genius can be recognized; he could not be referred to as a scientist of great erudition but his creative imagination and daring in the development of hypotheses which fitted the facts were the key to his success. Professor Kapitza thought that young people ought to read more of the original works of the great pioneers in science, and that this was especially important for those who are likely to be in charge of a group of

research students and who may have to select staff.

Lord Blackett was prevented by illness from attending the celebrations and his contribution was read by Professor N. Feather (University of Edinburgh). He reviewed Rutherford's remarkable career of scientific research and discovery and suggested ways by which a study of this can help science today. He reminded the audience that some of Rutherford's greatest discoveries arose from a keen observation of what may be described as chance or anomalous effects. Air draughts in his room at McGill University spoiled measurements of the strength of a thorium source but led to the discovery of the "emanation" gas escaping from the thorium and thence to the whole pattern of radioactive decay; a few alpha-particles coming back from the same side of a thin metal foil as was bombarded by a strong beam of such particles puzzled him and led him to what is generally regarded as his greatest discovery, the nuclear theory of the atom; and a few exceptionally long-range protons projected forwards from some light nuclei, but not from all, when bombarded by energetic alpha-particles led to the discovery of the artificial disintegration of nitrogen. "May every young scientist remember (these incidents) and not fail to keep his eyes open, for the possibility that an irritating failure of his apparatus to give consistent or unexpected results may... conceal an important discovery."

Lord Blackett himself had to examine 400,000 alpha-particle tracks to discover only six which revealed the process of disintegration described earlier by Rutherford. In those eventful years Rutherford was right in believing that nuclear physics was one of the greatest if not the greatest single branch of physics and he tended to decry the importance of some of the other branches, notably theoretical physics; he did not see as clearly as he might have done the impact of the study of the electronic structure of the atom on the developing quantum theory which itself was soon to supply the explanation of the range of the alpha-particle. Rutherford was not a mathematician but he used mathematics to great effect in his two greatest experimental achievements and he sought out those fields of research in which little mathematical reasoning was needed. He would have thoroughly enjoyed the early days of the new unstable elementary particles.

Professor Feather then described his own experiences in the Cavendish Laboratory from 1926 up to the date of Rutherford's death; he began as a research student and, apart from two brief interludes, he remained as a member of the staff and was perhaps the last to talk to Rutherford about the work of the laboratory before Rutherford went to hospital for the hernia operation from which he never recovered. Feather's research work for many years involved the use of the Wilson cloud



Professor P. Kapitza addressing the Rutherford colloquium at the thirteenth international congress of the history of science. On Kapitza's right, his secretary, Dr D. Shoenberg, Dr T. E. Allibone and Dr W. B. Lewis; on his left, Professor N. Feather, Professor S. Devons, Mr G. Danin and the chairman of the congress, Professor B. M. Kedrov.

chamber which was operated in those days most laboriously by hand and needed the assistance of a collaborator. During some of those years Rutherford, as president of the Royal Society, had many calls upon his time so his tours of the laboratory were less frequent than formerly, but Professor Feather described an experience shared by everybody at the Cavendish, of hearing Rutherford's heavy footsteps coming along the corridor, and his voice mounting louder as, in room by room, he fired questions about the work in progress, sometimes administering a much-needed prod to "cut out the frills", sometimes half grudgingly applauding good work, always cautious in assessing results, but then when quite satisfied, ensuring prompt publication as in the case of the papers submitted to the Royal Society announcing the discovery of the neutron and its effects.

Professor S. Devons (Columbia University), a product of the last phase of Rutherford's life, gave a vivid description of the undergraduate's contacts with the great men of that age, and then, later, attendance at the meeting of the Cavendish Physical Society. "Rutherford took the chair but was no passive chairman, his vigorous and amiable personality shone as he introduced the lecturer and later opened the discussion; his attitude was always unambiguously expressed." Facts were to be respected and treated differently from theory; though Rutherford greatly respected at least some of the theoreticians he disliked a smoke-screen of verbiage. The era of simple apparatus, the "string and sealingwax" trademark of the Cavendish was slowly passing when Devons began research—it had indeed been almost Henry Cavendish's own trademark 150 years earlier—but the budding research student still had to be "broken in" in the famous attic, making apparatus without assistance, using only the scraps and junk discarded in days gone by. His own choice of work, cosmic rays, had never actively interested Rutherford, possibly, Professor Devons suggested, because they were too complex and, not being under direct control in the laboratory, may have needed apparatus too elaborate for those days, and, as Rutherford had commented, the interpretation of results could prove a trap for the unwary.

Dr D. Shoenberg (University of Cambridge) contributed some delightful asides. Rutherford had once said that science can be classified into physics and "stamp collecting", but, as Shoenberg pointed out "stamp collecting" can yield rewarding results if a general principle can be deduced from the collection of data and Moseley's idea of making a systematic study of the X-ray spectra of the elements did indeed provide an important clue to the nucleus. Dr Shoenberg reminded the audience

that Rutherford won the Nobel Prize for "stamp collecting", that is, for chemistry, and on one occasion Rutherford introduced Debye with the remark "although he is a chemist he is really quite a decent sort of chap"!

Dr Shoenberg began his research as one of Professor Kapitza's pupils in the new building, the Mond Laboratory then being erected to rehouse Kapitza's intense magnetic field work and the new work on very low temperatures. Rutherford had taken a very great interest in the magnetic field work and had obtained for several years very considerable financial support for it, certainly considerable as judged by the standards prevailing in the "old" Cavendish. Although Rutherford was no expert in these two subjects studied in the Mond he had an uncanny way of getting straight to the heart of the matter under discussion, seeing what was missing, and asking for key measurements to be taken; and when Dr Shoenberg used mathematical terminology to describe the new theories of metals, Rutherford would say "now just tell me in simple language what that term is that you keep using".

Dr T. E. Allibone spoke of Rutherford's lectures at the Royal Institution and of his visits to the Royal Society Dining Club at which so many eminent Russian scientists had dined starting with Count Razumovski, the president of the Russian Academy who had dined in 1766 with Henry Cavendish, Benjamin Franklin and others. Rutherford was invited to give the Bakerian Lecture of the Royal Society in 1904, a signal honour for so young a man, he dined as a guest of the club that evening, and on the following day gave his first Friday Evening Discourse at the Royal Institution. It was there that he spoke of the energy released by the radioactive elements probably being responsible for a much slower rate of cooling of the Earth than had been calculated by Lord Kelvin—who was in the audience—a few thousand million years compared with Kelvin's estimate of a hundred million years. During the Manchester period he gave several discourses, demonstrating the counting of alpha-particles and describing the disintegration of nitrogen. His lectures were always very popular. Dr Allibone thought it doubtful that Rutherford ever believed in the theory of nuclear structure put forward by Gamow in 1928/29 which precipitated the work on positive-ion acceleration to half a million volts, because when Rutherford opened the new High Voltage Laboratory of the Metropolitan-Vickers Company in February 1930 he asked for millions and millions of volts and a discharge tube compressed into a small-sized room, whereas, if Gamow were to be believed Rutherford ought to have

asked for a very large current of positive ions accelerated to only a modest voltage.

Dr W. B. Lewis (Atomic Energy of Canada Ltd) spoke as one of the small band of personal collaborators of Rutherford. Most of the Cavendish men worked either independently or in pairs, responsible to one of the senior members of staff and only a few worked with Rutherford. Dr Lewis spoke of the strict routine to which Rutherford worked and expected his assistants to copy, a routine which had probably contributed greatly to his own success; "stop work at 6 o'clock and go home and think". In a short time this group of which Dr Lewis was a member had unravelled the complexities of the long-range alpha-particles, a subject which had attracted Rutherford's interest for a long time. The success was due to the use of the first of the new generation of "expensive" pieces of apparatus, a magnet designed and made for £250 by the M-V Company. After this Rutherford switched to his last experimental work, the disintegrations caused by deuterium ions accelerated to the paltry voltage of 200-thousand volts, in deference, so to speak, to Gamow's theory. He complained to Allibone about the cost of the 100-thousand volt transformer which the Metropolitan-Vickers Company had supplied. Allibone calculated that it had only cost a farthing a volt, cheaper than the cost of flash lamp batteries in those days, and Rutherford took the repartee in good grace.

Mr G. Danin, a well known Soviet writer on the history of science, discussed the role of Rutherford in the quantum interpretation of the planetary electrons. Published letters and material still unpublished in Copenhagen reveal a complex picture of collaboration between Rutherford and Bohr between April 1912 and March 1913; Rutherford's stimulating encouragement of a man in whom he had great faith combined with a restraining touch "take your time", "don't hurry". Mr Danin spoke of Rutherford's own unpublished attempts to find a quantum solution to the problem of the stability of the planetary model and he concluded that a thorough examination of all the papers and correspondence in the Manchester period would reveal much of interest to the history of one of the most fundamental discoveries of the century.

As each speaker left the rostrum Professor Kapitza presented him with a medal which had been struck in the Soviet Mint to commemorate the centenary, and also with it the first volume of Rutherford's collected works printed in Russian. Dr Allibone also presented to Professor Kapitza on behalf of the Royal Society of New Zealand the medal struck in New Zealand.

T. E. A.

Has Science Never Had It So Good?

by our Special Correspondent

ARE science and society now in a radical confrontation or does their apparent conflict merely represent old issues in new guises? This central question ran through the three controversial themes which dominated the Roche Jubilee Symposium, "The Challenge of Life—Biomedical Progress and Human Values", which was held in Basle from August 31 to September 3. What are the ethical responsibilities which scientists and doctors should assume in response to the advance of biomedical research? Should science and technology be more closely directed, perhaps even restrained, and if so, how? And how are the difficulties of communication between scientists and between scientist and layman to be resolved?

"Humanity is at a hinge of history", said Dr Philip Handler (President, US National Academy of Sciences), when introducing the first session, on biomedical frontiers. But his insistence that the rewards of science have created a critical situation, on whose resolution will depend whether man is a blind alley in evolution, was challenged by Solly Lord Zuckerman (former Chief Scientific Adviser to the British Government) who maintained that: "This is no new issue. This is the oldest issue I know . . . I don't believe for one second that this is a hinge of history. There have been corresponding hinges before. There have been people who hated the development of science and said so before." This disagreement, which was to be repeated during later sessions, foreshadowed the quarrel between idealism and pragmatism which the symposium spotlighted.

Molecular Biology: Threat or Promise?

Assessing the prospects opened for man by the dramatic advances of the past decade in molecular biology, immunology and neurobiology was the task of the opening speakers. Although there is general concern about the implications for man's heredity of the possible development of means for genetic manipulation, Dr Joshua Lederberg (Professor of Genetics, Stanford University) felt that the prophecies of genetic engineering merely bring into sharper focus problems which have been present for some time. There was also general agreement that organ transplantations are exaggerating ethical concerns which are in any case raised by medical immunology.

Particular controversy was aroused by the fear that it may become possible to clone humans to yield many identical individuals, with Dr Salvador Luria (Sedgwick Professor of Biology, Massachusetts Institute of Technology) saying that this idea is of as little scientific interest as the exploration of space; nothing could be learnt from it, he maintained, which could not be found by using the mouse instead. But Professor Lederberg felt that cloning humans would be interesting from another viewpoint, because of how it might help distinguish the effects of environment and heredity on man. Because cloning cattle, for example, might have useful implications for agriculture, Professor Handler asked whether perhaps it would be wise to take a decision on whether to support such research before a technique is developed which could easily be extended to man. The general view of the meeting, which was sanguine that on balance there is only slight risk that genetic manipulations or neurobiological techniques will be abused to change man, was caught by Dr Seymour Kety (Professor of Psychiatry, Harvard Medical School), who noted

that identical twins are not deliberately separated at birth to facilitate studies of the interaction of environment and heredity.

Gazing into crystal balls is always a hazard for the scientist, but when asked for predictions of future developments in the biomedical area, Professor Kety thought it likely that the major psychoses will be understood if not eradicated, and Dr Niels Jerne (Director, Basel Institute for Immunology) suggested that allergies and leukaemias are likely to be brought within the scope of medical treatment. Professors Luria and Lederberg agreed that molecular biology has as yet contributed little to modern medicine, but each predicted that it will prove indispensable as the basis of future treatments. Professor Luria specified the intensive research into reverse transcription, which he thinks is likely to be productive in understanding at least some forms of cancer; Professor Lederberg pointed to the extensions which seem likely in the scope of antenatal diagnoses.

The first topic on anyone's list for useful future research should be ageing, said Dr Arnold Burgen (Director, National Institute for Medical Research, Mill Hill), later during the symposium. This should include attempts both to increase the human life span and also to make the later part of life more liveable, although this latter problem is particularly difficult because of the way in which the different systems of the body age at different rates. Although endogenous diseases such as ageing seem likely to prove far more difficult to resolve than those caused by external agents, Professor Burgen feels that significant progress could be made if only a fraction of the funds supporting cancer research were put into such studies. Increases in life span which have already been achieved, however, were identified by Dr Alfred Sauvy (Professor of Social Demography, College of France) as the principal cause of the rise in world population. He is sure that future research will lead to some further abatement, even if it does not yield the dramatic lifespans of 90 or 120 years forecast by some people.

Over population was a topic which had loomed over all the sessions, said Alexander Lord Todd (Professor of Organic Chemistry, University of Cambridge) in his summary of the meeting. He took the consensus to be that Malthusian forebodings of famine are misplaced, but he supported Professor Handler's plea that although man has always had a sense of crisis, this time is different because the resources of the planet are only finite and some are beginning to run out. This contrasted with the earlier remarks of Dr Margaret Mead



Philip Handler.



Solly Lord Zuckerman.



Salvador Luria.



Joshua Lederberg.

(Professor of Anthropology, the American Museum of Natural History, New York) that some people regard population as an evil so great that almost any step should be encouraged to limit it, even if that step were drastically to change the nature of human society. The general opinion of the meeting, however, that society will in any case be changed, greatly for the worse, unless some acceptable way is found to limit population, was echoed by Professor Luria's comment that scientists and doctors have not fulfilled their responsibility to explain the consequences of population growth.

How Should Scientists Report Results?

The purposes which scientists ascribe to research were attacked by Dr Jeanne Hersch (Professor of Philosophy, University of Geneva), who argued that science is itself a vested interest, and that what this meeting really represented was the gathering of an élite of scientists to defend their interest. Promising a cure for cancer is just a ruse to keep funds flowing, she said; the molecular biologist working on "cancer" should recognize that his real motivation is not to cure the disease but rather to pursue truth for its own sake—and he should convey this attitude to others instead of using pretexts to gain funds. But the pursuit of science is in itself a human value and as such needs no further justification, insisted Professor Jerne, who extrapolated this reasoning to suggest that because social evolution may retard and perhaps even stop science, possibly quite soon, those people interested in research should press ahead as fast as possible while there is still time to do so.

The conservative view of science was taken further by Professor Jerne when he argued that scientists have already evolved the most apposite way to communicate their results and that no change should be made in this procedure. Their proper *modus operandi* is to publish original research articles; they should not try to popularize their work because it is philosophically impossible for any such attempt to avoid introducing some degree of distortion. Those who want to follow the conclusions of scientists should learn the language of science, he said. But other participants evidently felt that this attitude is symptomatic of the arrogance which tends to bring science into disrepute with society. The general consensus was with Dr Charles Berry (Director of Medical Research and Operations, NASA), who feels it is vital that scientists should explain their work to the public, and with Professor Lederberg, who stressed how essential it is to reverse the alienation of science for society. The scientist is responsible, he argued, to the community of people from whom he derives the funds which support his work.

There should be two levels of communication of scientific result, suggested Professor Burgen, one for scientists who may wish to repeat a particular set of experiments, and another to enable non-scientists to appreciate the significance of a research result if not the precise means by which it was obtained. Both

Professor Burgen and Professor Lederberg think that the communication network by which scientists communicate with each other works quite well on the whole, and Professor Burgen suggested that a similar communication network should be developed to enable scientist to communicate with non-scientist. Professor Lederberg feels that one way to approach this problem might be to make use of the facilities of the professional associations of scientists; these learned societies would be in a good position to provide accurate assessments of research.

Is there a Right to Health?

The conventional view that individuals are entitled to health was challenged by Professor Hersch, who suggested that health implies the ability to withstand minor ailments, which should not be subject to exhaustive treatment as they tend to be at present. When the improvement of health becomes an obsession, she maintained, it becomes an ill in itself. Of obvious relevance to the pharmaceutical industry were the discussions by Professor Berry, Dr Kenneth Arrow (Professor of Economics, Harvard University) and Dr Axel Strøm (Professor of Social Medicine, University of Oslo) of the attitudes of doctors and of how medical care is organized. Max Lord Rosenheim (President, Royal College of Physicians of London) summed up the general view that although new treatments and improved methods of diagnosis will change much of the organization of the medical profession, it is essential that the one doctor-one patient relationship should be maintained.

"The relief of minor symptoms is not only desirable from the point of view of the sufferer but also of the pharmaceutical industry", said Lord Rosenheim when answering one of the questions raised at the beginning of the symposium by Professor Alfred Pletscher (Director of Research, Hoffman-La-Roche): how much effort should the pharmaceutical industry devote to producing drugs to cure minor ailments? Lord Rosenheim's point was that the industry derives much of its income from just such drugs, and it needs the money made in this way if it is to be able to support the development of much more expensive, although less commercially rewarding drugs such as immunosuppressive agents. But in any case, Lord Todd said during his summary, even when the industry has decided how to balance its research efforts, drugs to treat minor ills will inevitably be developed as the result of side effects noticed during research originally designed for other ends. Another problem of some concern, that of the rising extent of self diagnosis and treatment, will also inevitably continue to increase, he observed, with the rising level of education of the population.

The demanding pattern of development of medical research was the concern of Lord Zuckerman's paper. Quoting a former British Minister of Health, Enoch Powell, he pointed out that demand for medical care is for all practical purposes unlimited. "Every advance in medical science creates new



Margaret Mead.



Alexander Lord Todd.

needs that did not exist until the means of meeting them came into existence, or at least into the realm of the possible. For every heart-lung machine or artificial kidney in operation, there must be many times that number of cases to which the treatment would be applicable." As Lord Zuckerman himself argued: "If money is provided for medical research workers, the brilliant research worker will use that money successfully. He will provide some new discovery, the discovery will be applied, and then you get the pattern of demand for the particular advance he has made". Continuing this reasoning, Lord Zuckerman argued that because resources must always be inadequate to meet demand, priorities must be imposed on the provision of treatment. Lord Rosenheim added that the provision of expensive treatments in early days must be limited not only by money but also by provision of trained doctors.

The inconsistencies in the pattern of medical care were remarked by Lord Rosenheim. "Contraception and abortion are preventing the life of an infinite number of healthy people, whilst medicine is keeping alive idiots, hydrocephalics and cases of spina bifida." The medical profession will not change this paradox, he said, which can only be influenced by an informed consensus of public opinion. And as Lord Zuckerman observed: "There is an unquestioned right to health and people have been brought up to believe they have that right. However scientific a treatment may be, it cannot hold its place in the market if there is no demand for it. Nor can the greatest quackery be kept off the market if there is a demand for it."

Should Biomedical Research be Curtailed?

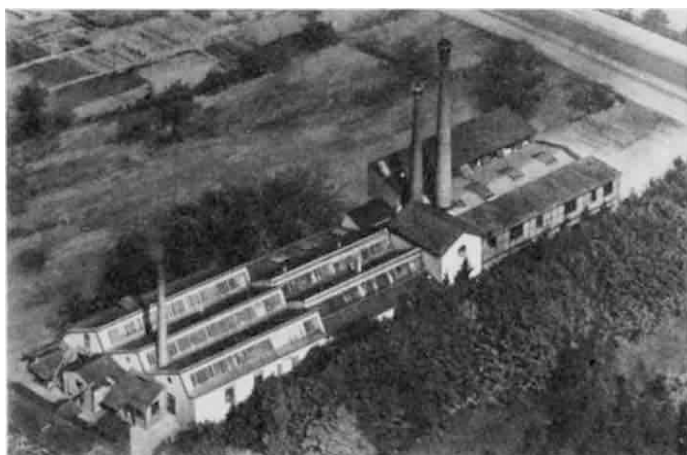
Is it irresponsible to allow advances in biomedical research to create demands which cannot be fulfilled? Should some of the funds now allotted to basic research be redirected to support studies of more clearly defined and perhaps more urgent needs? These talking points created considerable controversy. Lord Zuckerman's view was clear: "There can be no question in my mind but that the resources available will be inadequate for the claims of researchers. I am one of those who believe that (one should) beam research funds at certain fields in relation to need."

Both the ethos and the practicality of Lord Zuckerman's ideas were fiercely challenged by Professor Handler. First of all, he pointed out, many diseases which were at one time expensive to treat have now been eliminated completely. The patients who take great resources are those who have contracted diseases of this type which should by now have been eliminated,

or diseases for which only supportive rather than definitive treatments are available. The development of expensive treatments such as renal dialysis is just a stage, but a necessary one, on the way to developing a simple and inexpensive cure. The doctors present agreed that routine operations may develop from what is at first an operative technique used only at great expense in individual cases. Both Professor Handler and Professor Luria observed that, in any case, the cost of biomedical research in the United States (quoted as \$2.5 billion) is so slight compared with the cost of corrective medicine (quoted as \$70 billion) that it is illusory to suppose that there could be any appreciable financial saving from cutting back funds for biomedical research.

Opinions varied on whether society should control the direction of basic research in more detail, but the general consensus was that this is neither desirable nor, indeed, possible, because of the adventitious way in which knowledge advances. Because the motivation of the brilliant researcher who makes the more significant advances is intellectual curiosity, attempts to direct his efforts would be misplaced and unproductive. But the speed of change worried Professor Luria, who felt that we are engaged in a "breathless race with technology" because science and technology progress so much faster than the changes in the values of society which are needed to assimilate new living conditions. But an important distinction should be made, urged Professor Luria, between discovering new facts of nature and developing them into a technology. The critical stage at which to exercise control must be at the development of a technology, because it is not possible to control the development of research itself, nor to control the uses to which a technology is put once it has been developed. And because the acquisition of knowledge is fast in comparison with its application, even a moratorium on research would not halt the development of new medical treatments from existing knowledge for a considerable time yet.

The sense of the meeting was caught by Professor Handler when he argued that because only governments can now afford to pay the bill for research, and because resources are only limited, decisions on general priorities must be taken. Whereas it is an absolute necessity to decide how much goes into biomedical research it would be a very grave mistake to distinguish between the narrower parts of the biomedical sciences. Responding in particular to Lord Zuckerman's comments, he said that "if you mean anything narrower than that cancer research should go into the general properties of cells, you are wrong". Another point was caught by Dr Talcott Parsons (Professor of Sociology, Harvard University) who suggested that it might be of more use to control how



The first factory of Hoffman-La Roche in Basle, pictured in 1903, is shown on the left; the present buildings on the same site are shown on the right.

research is to be done, by setting standards of scientific competence and objectivity, than to control what research is performed.

Should the Sociologist Judge Science?

Although Dr Handler's eloquent assessment received vigorous applause from the meeting, the sociologists and theologians did not agree with it. Dr Franz Böckle (Professor of Moral Theology, University of Bonn) argued that the desire to knowledge must be subjected to certain limitations because it is impossible for the researcher to have a proper grasp of the overall situation. The real problem, he and Dr Jürgen Moltmann (Professor of Systematic Theology, University of Tübingen) argued, is to establish a set of ethics by which to judge biomedical progress, but for which the natural sciences alone cannot suffice and which must depend on the humanities.

This plea was echoed during a more detailed discussion of how research funds should be allocated. Dr Bernard Barber (Professor of Sociology, Columbia University), commenting on the peer review system by which panels of scientists assess for the US National Institutes of Health the values of projects in their own fields of competence, urged strongly that the sociologist, if not to be allowed to sit in judgment on the scientist, should at least play an important part in deciding the lines along which research should be directed. People who know the views of the public, he felt, should have at least some say in the allocation of funds. Professor Mead supported Professor Hersch's comment that scientists are too much an élite concerned only with their own problems, and complained that the accurate predictions which sociologists had made in the past that there would be a backlash to science had been ignored. An urgent need, she said, is for proper studies of the effects of scientific advance on the attitudes of society.

In contrast to the sociologists, Professor Luria is not too worried about the operation of the peer review system in the United States, but pointed to the discrepancy that although basic research is usually assessed by this means, its technological development is ultimately at the whim of the politician. Professor Luria intends to argue strongly at congressional hearings to be held in Washington next month that the new cancer research programme should be directed under peer review.

"I am frightened of peer review," said Lord Zuckerman, "because I know of peer reviews where there is only one peer, or two or three men." In a scathing attack on the way support for research is organized, he said he is particularly perturbed by the ways in which fashions in science can dictate the pattern of research. Professor Handler maintained that many of the options open to man have been generated by research, but according to Lord Zuckerman, "whilst science opens enormous avenues for the future, at the same time it is closing other options by setting fashions". Molecular biology is a notable example of this trend, he implied; in reference to Sir Macfarlane Burnet's recent view (in *Genes, Dreams and Reality*) that molecular biology has contributed little to medicine nor is likely to, he said that: "If six other Burnet's were to share that view, the Medical Research Council should start diverting funds to fields which represent clearly defined needs (such as) the problems of old age . . . which are receiving too little attention".

But would not setting priorities impinge on the freedom of the researcher? "We are already imposing the pattern of social justice on the pattern of medical research and it does not in any way infringe the independence of the medical research worker." Who should decide such priorities? "I dismiss (the medical research worker) because he merely creates demand regardless of need. I dismiss the public and client because he absorbs anything he is given." This left government, which, said Lord Zuckerman, was not desirable either. Referring in particular to medical research, he demanded that peers conducting reviews should hold a wider view of the field than just their own speciality. "Those people who have

a sophisticated view about resources and need should choose." *Sed quis custodiet ipsos custodes?*

The problem which Professor Lederberg visualizes is how to improve the social utility of research without damaging other values. But researchers have no less social conscience and probably more compassion than politicians, he suggested, and a "shopping list" made for useful research would in any case very largely list the present pattern of activities. Centralized planning, he argued, would be very likely merely to lead to the excesses of the space programme. Both he and Lord Zuckerman observed, however, that the scientist as such has no particular political franchise; if scientists and doctors wish to be the people who determine the ends to which research is put, they must enter directly into the political arena.

How Many Cultures?

Can the life sciences be integrated with the social sciences in some way? Professor Handler, summarizing his view of the meeting, said that: "All my life I have tried to be amongst those who have maintained, somewhat defensively I guess, that there are not two cultures". But he had rarely seen a better demonstration that there are indeed two cultures. Professor Mead was among those who felt that there had been too much talking and not enough listening. "The only way in which people from different disciplines can learn to understand each other is to look at the same problem."

But Professor Parsons suggested that integration can be provided by modes of thinking which go beyond one particular science, such as the idea of coding. Might there be some parallel between the biological and the linguistic codes, he asked? Lord Todd brought the meeting to earth by asking how integration might be achieved in practical terms, suggesting that perhaps the lack of interdisciplinary thinking is a consequence of the way university courses are organized. Professor Handler, however, maintained that integration of subjects occurs only in individual human brains; the failure of packages of arbitrary multidisciplinary at universities in the United States showed that there is no way to contrive integration. Professor Luria agreed that the function of a university is to give a superior training to people who can then use it to communicate effectively and to cross disciplinary boundaries.

Of what value did the conference prove to Hoffman-La-Roche? Introducing the meeting, Professor Alfred Pletscher said that "society has not considered soon and systematically enough the outcome and final consequences of biomedical advances. This omission must be remedied. The aim of the symposium should therefore be to define more clearly the problems created by biomedical progress and to find, if possible, essential facts or clues for their solution." At the end of the symposium, Professor Pletscher said that there could be no definite answer to most of the important questions, but that the symposium had succeeded in formulating more precisely the questions which should be asked. Now that a frame had been set to examine the problems, future conferences would be able to examine more specific problems.

The final discussion was directed by Lord Todd, chairman of the symposium, to answer the questions which Professor Pletscher posed at the outset. No certain way could be seen to avoid the potential harmful effects of research on mankind, but there was a consensus that fears are in general exaggerated. Basic research should not be directed, except in a very general way, and Professor Luria's point was well taken that the only option for control is at the development of a technology. An important target for biomedical research should be ageing, although this will bring other problems in train. And there was general agreement with Professor Burgen's refusal to regard the improvement of health as two edged. In spite of much talk, the most cogent comment on the ethical issues raised by biomedical progress was the lack of an answer to Lord Todd's question: "Can we harmonize biomedical science and human values?"

Science and the Common Market

BRIAN FLOWERS

State House, High Holborn, London, WC1

This article is based on a speech delivered by Professor Sir Brian Flowers, chairman of the Science Research Council, on September 6, 1971, at the meeting of the British Association in Swansea.

IN the great debate on the proposed entry of the United Kingdom into the Common Market science has been mentioned hardly at all, and even technology has received only scant attention to bolster up the economic arguments. It would be strange indeed if we were not to explore the likely effects of joining our West European friends on the advancement of science in this country and in Europe. I therefore propose briefly to do so.

But first I must make a declaration. I am a public servant appointed to a non-political post under the Labour government and now serving under a Conservative government and I must therefore make clear that I do not seek to indulge in party politics, nor am I acting, in this article, as a mouth-piece for the Science Research Council, still less for the government. I had hoped—together, I am sure, with many other people—that the Common Market issue would not be one to divide the parties, but alas that was not to be. For that reason the excellent custom whereby public servants do not indulge in party political controversy are best maintained if I confine myself as far as possible to examining the issues as they seem to me to concern science and technology.

Two Freedoms

I think we all accept that the two most basic elements of international science are the free exchange of information and the free movement of scientists across national frontiers. Indeed, one recalls the unhampered travels of Davy and Faraday in continental Europe during the Napoleonic wars. The learned and professional societies, as well as institutions like the British Association, contrive to preserve these international elements in spite of limitations inevitably imposed from time to time by national security and commercial confidence. In recent years the Royal Society with its post-doctoral exchange scheme has been particularly successful in the European context.

It is, however, a matter of quite recent history that the funding of science is also becoming international. This is the consequence of the scale on which some of it must be practised if anything significant is to be achieved; I need mention only nuclear physics, space research and, perhaps in the future, molecular biology. And it is the consequence of the nature of some branches of science, which are international in content as well as in practice: there would, for example,

be very little point in trying to explain the British climate using only British data. Indeed, for some branches of science it is necessary to consider the planet as a whole, and so we have bodies like the World Weather Watch and the World Health Organization.

Of course, our experiences of European scientific organizations have been far from uniformly successful. At the one extreme we have CERN, the European Organization for Nuclear Research, which is universally acknowledged not only as a successful scientific venture but as an example of successful international collaboration in general. It was created in 1952 during the post-war wave of European idealism, and its existence has enabled scientists from its member states to regain their position at the forefront of a science that is still of profound significance. Ironically, the UK did not join CERN at its inception—we waited to see whether it would be a good thing. And we hesitated in 1968 when the question arose of taking the next great step in energy to 300 GeV. But again we eventually joined, recognizing not only scientific merit but also the political will of Europe. At the other end of the spectrum we have ELDO, the European Launcher Development Organization, founded in 1962 at the instigation of the UK because we wished to find a use for our own discarded defence rocket Blue Streak; the combined European launcher which resulted has so far failed dismally to put into orbit even a Krushchevian orange. Unlike the more or less federal CERN with its efforts concentrated on its accelerators in Geneva, ELDO is much more a loose confederation of national efforts and it is in competition with the attempts of several member states to achieve a purely national launcher capability, our own included until the recent cancellation of Black Arrow.

Between these two extremes comes ESRO, the European Space Research Organization, which, in spite of acute and still unresolved conflict between its member states over the essential purposes and the scale of effort of the organization, has achieved some notable successes in scientific research in space, and by so doing has enabled a European technology to develop which may soon make its mark in satellite applications such as regional telecommunications, meteorology and navigation. Nearer to the failure end, but this time innocent of British assistance, comes Euratom, the Atomic Energy Authority of the Common Market. Both of these organizations, but especially Euratom, are again in competition with national enterprises deemed to have greater success and significance. There are those who hope that the accession of the UK to the Common Market will bring in fresh air for Euratom to breathe; others believe that asphyxiation would be a better remedy in this case. Unlike the other organizations I have mentioned, Euratom is one of the official Common Market institutions and it will require very close examination in the future.

A more recent case is that of the still embryonic European Molecular Biology Organization, dedicated to exploit on a European scale one of the most exciting developments in all

of science, our recent chemical and physical understanding of the nature of biological processes at the molecular level. This began controversially not because of the quality or content of the science, but because of the scale on which it was originally proposed that it should be prosecuted. The attitude of the British scientific establishment at that time was hostile because of the scale and because we were already strong in the subject. On the scale now proposed, however, this is a case where, confident of our own achievements, we might now feel that we have something to give to the other nations of Europe in an enterprise whose effects may yet be still more significant to our understanding of the life sciences, including medicine.

Criteria for Success

The lessons to be learnt, it seems to me, from this brief survey of some of the existing European scientific and technological organizations are five-fold. First, the science must be good science and the purpose of setting up the organization must be clear; second, the initiative for creating the organization must come from the scientists working in the field; third, scientists of the highest quality in each country must want to take part; fourth, the nature of the science must render organization on a European scale either necessary or at least likely to bestow distinct benefit on all participants; and finally, each country must give its support of the organization priority over any residual national effort in the field. CERN has satisfied all these points; the other European organizations I have mentioned have not.

The internal criteria I have described for a successful European research organization seem stringent enough, but scientific research is only part of the whole social activity. There are also economic, political and social criteria which exert a powerful influence on what research is supported from public funds and on how that research is organized. Thus in fields where independent sovereign governments and business concerns believe that the results of research could give their possessor some economic or other social advantage, the pressure to support national programmes may be great enough to prevent successful international collaboration. This has been the case with space technology and with nuclear power. Indeed, attitudes towards collaboration in technology sometimes differ sharply from attitudes towards collaboration in science because of the hope of earlier gain. Even on a national scale there have been many examples of firms failing to collaborate wholeheartedly in government supported co-operative research projects, which could bring benefits to all, because they feared that their competitors might secure the greater benefit. In cases where research can be expected to lead to a fairly early economic or social benefit some obvious common interest is required to ensure real cooperation. For example, all countries are persuaded that it is in their interest to control plague, and realize that this must be tackled on an almost world-wide scale. In economic and political matters, however, our self-interest is not yet enlightened enough for the majority to see that cooperation both in research and in its applications will pay simply because, ultimately, we prosper or suffer together.

It will be a long hard slog to the millennium and, meanwhile, anything we can do to encourage real collaboration between sovereign groups will help enlightenment. Despite all the difficulties I have mentioned, scientific research is a very promising area in which to encourage international collaboration in general. Such collaboration, of course, took place in fundamental science long before the Common Market was thought up. No doubt this will continue if only to meet objectives beyond the scope of Western Europe. But the Common Market will provide a psychological as well as a financial framework within which international collaboration in Europe will become more naturally the rule than the exception.

What is Science?

To be able to discuss the effects of the Common Market upon science, we must first be clear about the nature and purposes of science.

Science in the first place is a cultural activity and, as such, peculiarly a product of European culture. It changes profoundly our comprehension of the world around us and of our place in it. We have only to remember the work of the early astronomers like Galileo and Kepler and of the impact of their ideas upon contemporary philosophical and theological thought, or of Darwin on the origin of species, or of Einstein on the nature of space and time, or of Rutherford on the structure of the atom to see that science is sedition at its best. Modern astronomy is striving for an understanding of the nature and origin of the universe, particle physics for the ultimate structure of matter and the forces which act upon it, molecular biology for the templates of heredity and the nature of biological laws. Their effect upon our understanding of the world about us is likely to be no less profound. It is even a tenable hypothesis that space travel, and the first glimpse it has given us of our world from outside, has helped to focus our attention on the problems that bedevil our sorry planet and its inhabitants. If so it will have paid for itself.

But there is more to it than that. Society demands satisfaction from its investments in science; it has goals it wishes to achieve. It wants better transport, better communications, better housing, better health, better food, more electric power; it wants to be defended, it wants more rewarding leisure, more relevant education: it wants all these things at a reasonable price, and increasingly it wants them not to intrude in unwelcome ways, not to pollute the environment with noise or filth or poison. But none of these things is possible—or at least they would be much more difficult to achieve—without science. For the great majority of people, whatever they may think of science in their leisure hours (if they think of it at all) and whatever its cultural value, science is not a thing in itself but a means to achieve some of the aims of society.

Counting the Cost

The goals I have mentioned are, by and large, recognized in every industrialized country in the world as so important that they each rate a separate government department. Many of them, indeed, can be pursued only on an international basis of some sort. They are not so much national goals as goals of the human race to be striven for independently of national politics, and in some cases in spite of national economies. It is in the hope of achieving these aims of society, and not for the sake of science itself, that the industrialized nations of the world devote something like 2% of their gross national product to research and development, including something like a tenth of that to fundamental scientific research. Scientists cannot expect support on this scale—indeed they should not have it—unless their work can be seen to form part of a pattern which as a whole produces substantial public benefits.

Of the science-based industries—those that depend especially upon automation, instrumentation, electronics, chemistry, and materials science—most are in favour of joining the Common Market. The reason is that many of them believe that a home market of 50 million people is insufficient as an export base, and that 300 million is nearer the mark. And, in turn, the reason for this is often that only on such a basis can the huge research and development overheads be covered in an economic manner—aerospace is the notorious example, but nuclear power and computers are others. And in some cases—transport and communications, for example—it is only by adoption of a much larger measure of standardization than is possible on a purely national basis that economic operations become possible, our recent moves towards metrication were, of course, a helpful step in this direction. As far as science is concerned, our post-

war failure to achieve a steady economic growth has been due not so much to any failings, real or imagined, in our achievements in pure and applied research, but to our inability, with a home market increasingly confined to the UK, since the loss of an empire, to find the wherewithal to pay the much heavier development costs (including prototype production) required to profit from expenditure on research.

On a European basis we can expect to be able to afford our share of these development costs provided that we, and our partners, gradually give up the idea of alternative and purely national competing ventures. And in streamlining our research and development efforts within the Western European nations we can afford to devote more than we have done in the past to mitigating some of the undesirable effects of technological growth—for the humane technological society requires more, not less, research.

The six are already working towards a common energy policy, in particular for cooperation between themselves and the oil-supplying countries. This, of course, is basic to all industrial activity. They hope to widen the field of trade with the oil countries and thus improve relations generally between themselves, the oil countries, and the oil companies. They also plan to take joint action to support exploration for new supplies of oil, and to raise loans to finance the building of nuclear power stations, a matter which I imagine will be of great importance to us. But improvement of the quality of life is just as important an objective of the economic and social policy of the community as the quantitative raising of the standard of living. Because pollution does not recognize frontiers and because no country can be expected to act alone to penalize its industries in competition with the less scrupulous, action must be taken by the community as a whole. Joint action has already been proposed to control air and water pollution. The community intends, for example, to support and extend the work of the International Commission for Rhine Pollution Control in order to protect the North Sea as well as the whole of the Rhine basin.

Facing the Competition

The need for a larger home market is perhaps arguable; some would say that it exists already in spite of tariffs. But excessive competition between countries of limited industrial capability reduces the share of the world market available to each. The real competition is very often with the super-states, especially the United States and, increasingly, Japan, and it is in this context that European cooperation can be most effective. I believe that this applies to research and development as much as to production. The result of joining the Common Market can be expected to be a much larger measure of rationalization amongst European firms, some of it painful in the short term. We may complain about the common agricultural policy as a device to protect inefficient European farmers, but we should not forget how many of our own industries are at present inefficient, and depend upon protection of one sort or another in order to survive foreign competition. The multinational industrial corporation may be expected to take the place, to some extent, of national protectionism. There is also great value for both science and technology in having common tasks tackled by teams of mixed nationality, provided that one can extract the best from the varied experience and training of the different nationalities. We have surely all learned by now that British is not always best!

Especially if the social goals I have mentioned are to be achieved, an industrialized and free society like ours can neither afford to go it alone nor can it, in my view, allow itself to become utterly dependent upon a super-state, whether in the east or the west, in which it has no hope of sharing in and contributing to the government. It is better that we join a community of like-minded nations of similar size amongst which our voice must and will be heard. Nor can we allow ourselves to become politically and economically subject to the uncontrolled

growth of multi-national corporations which at present owe allegiance to no nation and whose annual turnover in some cases already exceeds the individual gross national products of several European nations. Thus, regardless of whether it is international capitalism or international socialism that we wish to foster—and because we live in a mixed society it may be that we should wish to foster both—we have more hope of achieving an acceptable result within a community of like-minded nations especially when, in both respects, they have as much to offer to us as we have to them.

Watching Over Science

We are much exercised in this country at the present time with trying to match the inner logic of scientific activity to the aims of our society. In this endeavour the research councils have a vital function, for it is primarily their responsibility to look across the whole range of science from astronomy to production engineering, from molecular biology to social behaviour, from botany to meteorology, to see not only that society provides for the needs of its scientists, but that the pattern of scientific research and the training of scientists and technologists in the universities and colleges adapt themselves as time goes on to the changing needs of society. From time to time governments ask themselves whether the research councils should have as much independence as they have at present. Certainly adjustments need to be made at intervals, but the fact is that most countries decide to channel a proportion of their support for research through organizations which are not themselves responsible for the achievement of specific applications. Whatever failings our research council system may possess it is still among the best in the world; it is something in which we can take some pride and something we can offer to others.

If we join the Common Market there will, of course, in time emerge a further layer of committees and consultations in the hierarchy which determines our scientific and technological affairs. This will take time to evolve, and it will bring with it new frustrations for those who feel that the national system is already over-burdened. Be sure it will also bring frustrations to those who have to operate the system! But we can take some comfort from the fact that European nations all seem to be dealing with the same kinds of problems in the same kinds of ways in spite of different national systems. In recent years there have in particular been many discussions between the research councils and similar bodies on the continent. Correspondingly, there has been a series of meetings at ministerial level which have attempted to define priorities in fields of technology which are of immediate concern. From all this can be expected to emerge, in some form at least, a consultative assembly of the various scientific authorities of Western Europe, a new forum for scientists and for governments to discuss issues requiring, for reasons of scale or of content, organized international coordination and funding, whether it be the provision of a new and expensive scientific facility, or the war against pollution, or the rationalization of industrial research and development.

If, as a result, our administrative procedures become more cumbersome and therefore move slow moving—and I fear that this is almost bound to be the case for a time—we must set this disadvantage against the fact that it will enable us to continue to take part in, and certainly in some cases to lead, activities which are rapidly growing beyond the capabilities of individual nations. There will not necessarily be any disadvantage in the longer term, because if we have learned anything from the past few years it is that science can only benefit from a deeper and wider discussion of its content and purpose.

This, for scientists, mirrors in a small way the problem of political sovereignty that worries so many people in this country. By sacrificing a certain measure of our own sovereignty, more often than not over matters which are in any

case beyond our sole control, we share in a wider sovereignty within which we can hope to achieve more. And, insofar as scientific affairs are determined somewhat differently in the various European countries, we have perhaps something to gain on that score also. Scientists, too, will have their analogues of the political and economic problems of the development regions. Each country has its scientific strengths and weaknesses and it will be important to see that, while maintaining the strengths, we do something to ameliorate the weaknesses, whether they be in scientific or in geographical areas.

More Collaboration

In recent years we have seen the advantages of university departments and other research institutions with parallel or complementary interests coordinating their activities in order to share more equitably in limited funds and to have access to facilities to which otherwise they can no longer singly aspire. This in microcosm, is the whole story. But even in microcosm it can be extended. There should be nothing to prevent the collaboration between departments of British universities and departments of other European universities, and in principle there is nothing to prevent it now. Indeed, the research councils are already trying to encourage such developments. It is clear that the only real barrier is a mental one: we are not yet, most of us, accustomed to thinking in such terms. Our work will be immeasurably enriched, both financially and intellectually when collaboration at this grass root level has become commonplace. From it will come not only advantages to science, but also a sharing of experiences between universities of different countries on a scale not yet seen. And here again we have something to offer in exchange, for there is a great deal to admire in the British university system.

It seems to me that much the same can be said of industry. Although there is already a measure of European collaboration between companies, we are not yet accustomed to thinking of it as commonplace, especially among smaller firms. The placing of joint Common Market development contracts would help to speed the processes of collaboration and rationalization without which an economic union is unlikely to succeed at the technical level.

But we shall have to be wary of undertaking scientific or technological ventures for purely political reasons, merely in order to appear European. Building up a new Europe is indeed a worthy end in itself, and one that scientists and others must be ready to accept. But we and our partners will be foolish to forget that, however high our purpose, bad science leads to bad politics and bad technology to bad economics.

The fear has often been expressed that the Common Market is an inward-looking community. This seems to me an extremely odd view of the efforts of a group of nations who have done at least as much as ourselves to foster contacts between east and west and whose record in helping the developing nations is as good as ours. And it comes singularly amiss from individuals who simultaneously insist upon the supposed advantages of our antiquated monetary system, now fortunately buried.

Be that as it may, in science there has so far been, on the whole, a recognition that exclusiveness is not the proper path. CERN was born before the Common Market and its membership has always been open to all the West European countries. It has observers from Poland, Turkey and Yugoslavia, and it has throughout maintained the closest of international working relationships especially with the United States and the Soviet Union. This will remain the case even when its membership is chiefly that of the enlarged Common Market. And in our space affairs, too, there has been the closest relationship with, and indeed a large measure of dependence upon, the United States. Enlightened self-interest as much as idealism is likely to preserve this state of affairs in a world becoming increasingly "the space-ship Earth".

Certainly we shall have to play our part in maintaining an outward-looking stance, and our world-wide bonds of common language will help us to do so. The profound historical and economic interests which the European countries, especially France, Holland and Britain, have in the developing nations of Asia and Africa should make possible an extensive attack on problems of tropical medicine and agriculture to which our contributions in this country have already been highly distinguished. The possibility of extending scientific research within the Common Market towards the solution of problems relevant to the developing world, such as the local development of natural resources, could make an important contribution to the relationship between Europe and most other countries.

Funding Science

Until about five years ago post-war science budgets had been growing at about 10 to 15% a year—at about the maximum rate, that is to say, which could be absorbed by the scientific community. It was a mark of confidence in the importance of scientific research and in the ability of trained scientists and engineers to use their talents in the service of the nation. Indeed, wartime experience had amply justified this view. Since then, and not only in this country, a certain measure of disillusion has set in. Our rapidly increasing expenditure on research has not led to the once expected increase in national wealth. We have begun to doubt whether new science leads to new technology, and even more whether new technology is synonymous with progress for the human race. We have been forced to ask whether our priorities were right, whether more effort should not be devoted to mitigating the effects of technological progress rather than to hastening it and whether an increase in the quality of life is not more important than sheer economic growth. And yet, at the end of the argument, the quality of life has to be paid for from the profits of technology, whatever the remedy may be in detail.

In our own case the process has been made more acute by our lack of many vital natural resources, by a home market insufficient by itself to support a highly technological export industry, and by the political vacuum resulting from a lost empire and a slowly deteriorating economic position. Of course we are better off than we were twenty years ago, but we have been slow to realize, in the fastness of our island state, that our competitors have been steadily gaining over us in their standard of living and in their influence in the world.

Modern science and technology can flourish only in the environment of a prosperous industrialized state; it is utterly dependent upon industry, not only for its share of the taxes contributed by firms, but for power supplies, instruments, materials and top-grade manufacture. If the nation is not prosperous, science cannot prosper; this is the immediate effect. But in the longer term, if science does not prosper the basis of a technological nation is undermined and it will never become prosperous. Scientists therefore have to be more outward looking than they have ever been except in time of war. We have to turn our minds more towards the scientific basis of the twenty-first century, more towards mitigating the effects of twentieth century technology and more towards the problems which have arisen for the human race from the technological advances we have ourselves helped to create, not least for the developing world. And we have to do it on a scale that we cannot by ourselves sustain.

If we join the Common Market we shall at least be presented with an opportunity—an opportunity to gain; an opportunity to contribute; an opportunity to refresh our culture and to regain our prosperity within a common European heritage. We shall be joining a community of nations less afflicted by disillusion, who have not lost their faith in the cultural value of science nor in the economic value of its application. It is an opportunity we must not fail to grasp.

Use of Frog Eggs and Oocytes for the Study of Messenger RNA and its Translation in Living Cells

J. B. GURDON, C. D. LANE & H. R. WOODLAND

Department of Zoology, South Parks Road, Oxford

G. MARBAIX

Department of Molecular Biology, University of Brussels, Rhode-St-Genèse

Injected frog eggs and oocytes provide a very sensitive assay system for the identification of messenger RNA and permit the study of translational control in living cells. The translation of each haemoglobin messenger RNA molecule once every 5–10 minutes for at least 24 hours makes it possible to recognize less than 1 ng of this messenger RNA.

EXPERIMENTS described elsewhere have shown that oocytes injected with haemin and purified 9S RNA from rabbit reticulocytes will synthesize haemoglobin; the effect is not obtained with other kinds of reticulocyte RNA^{1,2}. This raises the possibility that the microinjection of messenger RNA (mRNA) into frog oocytes and eggs may constitute a generally useful experimental system for identifying and studying the translation of different kinds of mRNA. This system has the unique advantage of enabling purified RNA to be translated and the control of this process to be studied in a normal living cell. In this article we discuss several of the conditions associated with the successful translation of injected mRNA. In particular we are concerned with the treatment of recipient cells, with the duration and efficiency of haemoglobin (Hb) message translation, and with the potential use of this experimental system for translating messages other than that for Hb.

9S mRNA for haemoglobin has been prepared by SDS treatment of the 14S messenger ribonucleoprotein released from reticulocyte polyribosomes by EDTA¹. For all experiments described here, oocytes and eggs were taken from *Xenopus laevis*. Except where stated, oocytes were taken from frogs which had been induced by hormone treatment to ovulate between 2 and 4 weeks previously. This was done to ensure that the larger oocytes were growing actively. The eggs used were unfertilized, but underwent the usual activation response to penetration. Oocytes were injected dry but without the removal of follicle cells², and eggs were irradiated

with ultraviolet light to facilitate penetration of the jelly and vitelline membrane³.

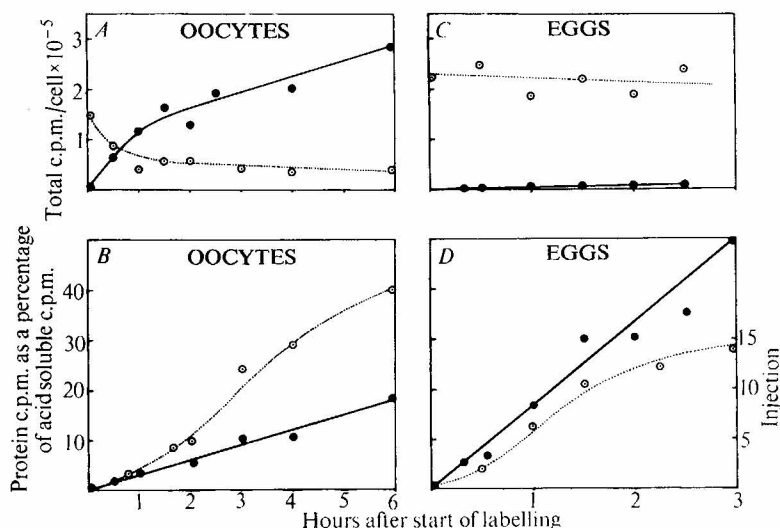
Micropipettes 10–15 μ m in diameter were calibrated to deliver a volume of 50–70 m μ l., the actual amount being kept constant in each related series of experiments. Samples for injection were kept on ice, as well as under oil to avoid concentration by evaporation. All samples were taken up in, or dialysed into, the following injection medium: 88 mM NaCl, 1.0 mM KCl, 15 mM Tris-HCl, pH 7.6. Injected cells were incubated at 19° C in the saline medium described by Gurdon².

Labelling of Eggs and Oocytes

Labelled amino-acids can be introduced into cells either by injection or by addition to the culture medium. The choice of labelling procedure is determined chiefly by: (a) the rate at which label in the culture medium penetrates incubated cells; (b) the rate at which injected label leaks out, and (c) the duration of the labelling period.

We have investigated the labelling of cells by ³H-histidine, with the results shown in Fig. 1A–D. Labelled amino-acids leak out of injected oocytes rapidly, so that less than 50% remains after 1 h, but leakage from injected eggs takes place at a much lower rate (Fig. 1A and C). In contrast, labelled histidine in the medium penetrates eggs very poorly, but is rapidly taken up by oocytes (Fig. 1A and C). The incorporation of intracellular amino-acid into protein is shown in Fig. 1B and D. The general conclusion from experiments of this kind is that when use is made of short labelling periods of up to 1 h or of unfertilized eggs (which do not normally remain metabolically active for more than a few hours), the highest yield of labelled protein is obtained by injection. When oocytes are used, especially for labelling periods of more than 2 h, it is best to label by incubation (at least for ³H-histidine). A minor disadvantage of labelling oocytes by incubation is that the follicle cells and very small oocytes (usually present in ovarian tissue containing large oocytes) will take up label and synthesize labelled "endogenous" protein, but not the protein coded for by added mRNA because they are too small to be conveniently injected. When it is important to eliminate totally the background contributed by follicle cells and small oocytes, unfertilized eggs (which have no follicle cells) should be used.

Fig. 1 *A* and *B*, Full size oocytes; *C* and *D*, ultra-violet irradiated unfertilized eggs. For incubation experiments (●) cells were injected with saline solution and incubated at 19° C in culture medium containing ³H-histidine at about 1 mCi/ml. For injection experiments (○) each cell received 50 nl. of ³H-histidine at about 6 mCi/ml. At the end of the labelling period, samples of ten cells were frozen. After homogenization in 0.5 M KCl 15 mM Tris, 0.1 % unlabelled histidine, pH 7.6, an aliquot was dissolved in 90 % formic acid and counted to give total counts per sample. The rest of the sample was precipitated in 10 % trichloroacetic acid (TCA), and centrifuged. The pellet was taken up in 10 % TCA, heated at 90° C for 30 min, pelleted, rewashed in cold TCA, then with alcohol and ether, and finally dissolved in 90 % formic acid for counting. From this was determined the amount of labelled protein in the original sample. In *A* and *C*, it is important to note that the total radioactivity includes protein as well as acid soluble c.p.m. The proportion of total radioactivity which is protein can be determined by reference to *B* and *D*.



Translation of Hb Message

The accumulation of labelled haemoglobin in conditions of continuous labelling is shown for injected eggs in Fig. 2*A*. The slow initial rise in the rate of Hb synthesis probably reflects the time needed (10–15 min) for the injected message to become fully incorporated into the structure of the cell in such a way as to serve for protein synthesis; the reduced rate at which labelled Hb is accumulated after 2 h from the time of injection can be partially accounted for by the substantial reduction in the amount of unincorporated labelled amino-acid which remains in eggs injected 2 h before (compare Fig. 1*C* and *D*). The translation of Hb mRNA is compared with that of endogenous mRNA in continuously labelled oocytes in Fig. 2*B*. The amount of Hb mRNA injected was sufficient in these experiments for the labelled Hb to amount to about half that of the labelled “endogenous” protein. It is clear from Fig. 2*B* that both Hb and endogenous protein accumulate at approximately constant rates during the 9 h period of these experiments.

The relative rates of Hb and endogenous message translation have been further examined by pulse labelling oocytes injected with Hb mRNA. Fig. 3 shows that the ratio of Hb to endogenous protein synthesis is similar up to 21 h after injection. Only within the next 10 h is some decrease in this ratio observed. Because no steps were taken to prevent RNA

synthesis which takes place in cultured injected oocytes², this last result does not necessarily indicate that the rate of translation or stability of Hb mRNA differs from that of the endogenous mRNA already present at the time of injection.

Table 1 summarizes some other respects in which injected and endogenous mRNA translation have been compared. Injected message translation is at least as sensitive as that of endogenous message to the inhibition of protein synthesis by puromycin. The intracellular location of injected message translation is interesting because the nucleus of normal oocytes always has a higher concentration of labelled protein than the cytoplasm, even after short labelling periods (discussed in ref. 4). It has not yet been clearly established whether proteins are synthesized in the nucleus or whether they pass from the cytoplasm to the nucleus very soon after synthesis. From the experiments summarized in Table 1, it seems that injected message is translated in the same part of the cell as most of the endogenous message. The fact that haemoglobin synthesis takes place in oocytes enucleated before RNA injection, as well as in unfertilized eggs which possess no nucleus but only ultraviolet-irradiated chromosomes, shows that Hb is synthesized largely, if not entirely, in the cytoplasm.

We conclude that injected Hb mRNA becomes rapidly associated with components of egg and oocyte cytoplasm so as to be translated in the same way as resident mRNA molecules already in use for protein synthesis. Once started, Hb

Table 1 Inhibitor Sensitivity and Intracellular Location of Hb Synthesis by Injected Oocytes

Recipient cells *	Materials injected	Protein synthesis as % of acid soluble radioactivity		Synthesis ratio: haemoglobin/endogenous
		Endogenous protein †	Haemoglobin	
Large oocytes	9S RNA at 800 µg/ml. and haemin	14.6	43.9	3.01
Small oocytes (half max. diam.)	9S RNA at 800 µg/ml. and haemin	12.5	41.6	3.33
Large oocytes	9S RNA at 800 µg/ml. and haemin	Puromycin at 700 µg/ml. 2.4	3.5	1.46
Large oocytes, enucleated after incubation ‡	9S RNA at 800 µg/ml. and haemin	10.1	33.3	3.30
Large oocytes, enucleated before injection ‡	9S RNA at 800 µg/ml. and haemin	1.5	6.4	4.27

* Each sample, which consisted of thirty oocytes, was labelled by incubation in ³H-histidine for 6 h at 19° C.

† Calculated from ‘Sephadex G-100’ fractionations as described by Moar *et al.*¹³.

‡ Enucleation involved manual removal of the germinal vesicle, which is squeezed out of oocytes after an animal pole incision. The oocytes injected after enucleation did not heal satisfactorily, and incorporation into protein was very low.

mRNA and endogenous mRNA are translated at the same relative rate for at least 21 h; Hb mRNA continues to be translated, possibly at a lower rate, for several hours after this.

Efficiency of Hb mRNA Translation

We wish to know the frequency with which each injected mRNA molecule is translated per minute; this information will permit an estimate of the total number of times an mRNA molecule is translated during its life in an egg or oocyte. The frequency of message translation can be determined from a knowledge of the number of mRNA molecules present in a host cell, and of the specific activity of the intracellular histidine pool (hence the number of Hb molecules synthesized).

The size of the histidine pool in eggs and oocytes has been determined by fractionating on an automatic amino-acid analyser the supernatant of cells homogenized in 0.5 N perchloric acid. One sample of 300 oocytes, from which the follicle cells and ovarian tissue were not removed, contained 36 pmol of histidine per large oocyte. Another sample of 400 oocytes, from which were removed all small oocytes and ovarian tissue except the follicle cells immediately surrounding each large oocyte, contained 23 pmol of histidine per oocyte. A sample of 350 unfertilized eggs, analysed in the same way, contained 91 pmol of histidine per cell. These values for the histidine pool may be compared with the figure of 146 pmol per egg obtained by Kutsy *et al.*⁵, using unfertilized eggs of *Rana pipiens*, and 330–700 pmol per egg obtained by Ecker and Smith⁶ for eggs of this same species. Since *Rana pipiens* eggs are three times the volume of *Xenopus laevis* eggs, our measurements suggest that a similar or slightly smaller intracellular concentration of histidine exists in *Xenopus* than in the only other amphibian species tested.

Oocytes from the same frogs as those used for estimating the histidine pool size have been injected with Hb mRNA, and the efficiency of message translation has been calculated according to the procedure outlined in Table 2.

To test whether the results summarized in Table 2 are representative of our normal experience, we have calculated in Table 3 the efficiency of translation of the same sample of mRNA used in fifteen other experiments with cells whose histidine pool size was not measured but assumed to be similar to that of other oocytes or eggs. The two samples of

oocytes which gave the results in Table 2 were evidently worse in one case, and better in the other case, than is typical. As might be expected, the efficiency of translation is greater at the lowest intracellular concentrations of injected mRNA. This effect is clearly seen in Fig. 4 and is consistent with the observation, reported elsewhere¹³, that there is a substantial decrease in translational efficiency at intracellular concentrations of message which exceed 8 µg/ml.

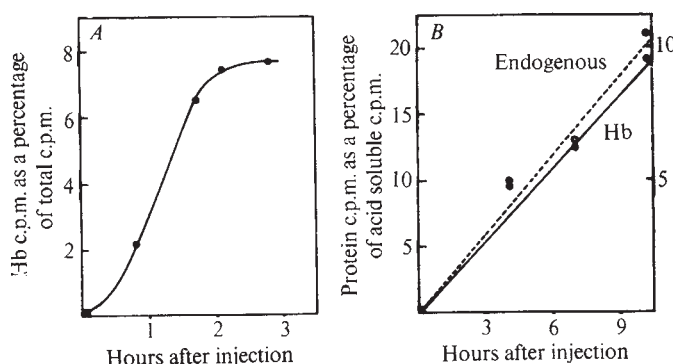


Fig. 2 A, Each point is based on a 'Sephadex' analysis of thirty unfertilized eggs injected with 9S RNA at about 800 µg/ml., haemin, and ³H-histidine, and frozen at the times shown. B, Each point is based on a 'Sephadex' analysis of twenty large oocytes injected with 9S RNA at about 800 µg/ml. and haemin, incubated in medium containing ³H-histidine, and frozen at the times shown. "Endogenous" refers to protein synthesized from endogenous mRNA, as opposed to the injected 9S mRNA. The ordinate on the left refers to endogenous and that on the right to Hb radioactivity.

In estimating the efficiency of translation, we have made certain assumptions which affect the accuracy of our calculations in the following ways. In determining the specific activity of the histidine pool, our measurements of the extractable free histidine constitute an upper limit to the size of the pool that is actually used for protein synthesis; this could have been

Table 2 Efficiency of Hb mRNA Translation in Injected Oocytes of Measured Histidine Pool Size

	Oocytes of frog A		Oocytes of frog B		
1 Concentration of Hb mRNA in sample for injection (µg/ml.)	200	250	125	62.5	31.25
2 Injected Hb mRNA (ng/cell) *	14,000	17,500	8,760	4,360	2,180
3 " " " (pmol/cell) †	0.070	0.087	0.043	0.022	0.011
4 Number cells/sample	30	20			
5 c.p.m. ³ H-histidine incorporated into Hb/cell/h ‡	4,480	6,428	8,490	6,439	3,155
6 Pool size of histidine (pmol/cell)	23.2	35.9			
7 Specific activity of intracellular histidine pool (c.p.m./pmol) §	2,845	758	1,392	2,654	1,186
8 Globin chain synthesis (pmol globin/cell/h)	0.157	0.848	0.610	0.243	0.266
9 Number globin molecules synthesized/h/9 S RNA molecule (row 8 ÷ row 3)	2.24	9.77	14.0	11.1	24.35

* These values disregard leakage from injected oocytes, though this is believed to occur (see text).

† Assumes that the molecular weight of 9S mRNA is 200,000. Various published and unpublished measurements by different methods have estimated it to be between 175,000 and 225,000 (for example, ref. 8).

‡ All samples were labelled by incubation for 10 h at 19° C in ³H-histidine of specific activity 52.1 Ci/mmol.

§ Calculated from the histidine pool size and the acid soluble c.p.m./cell recovered from columns of 'Sephadex'.

|| Assumes 9.5 histidine residues per average (α and β) globin chain.

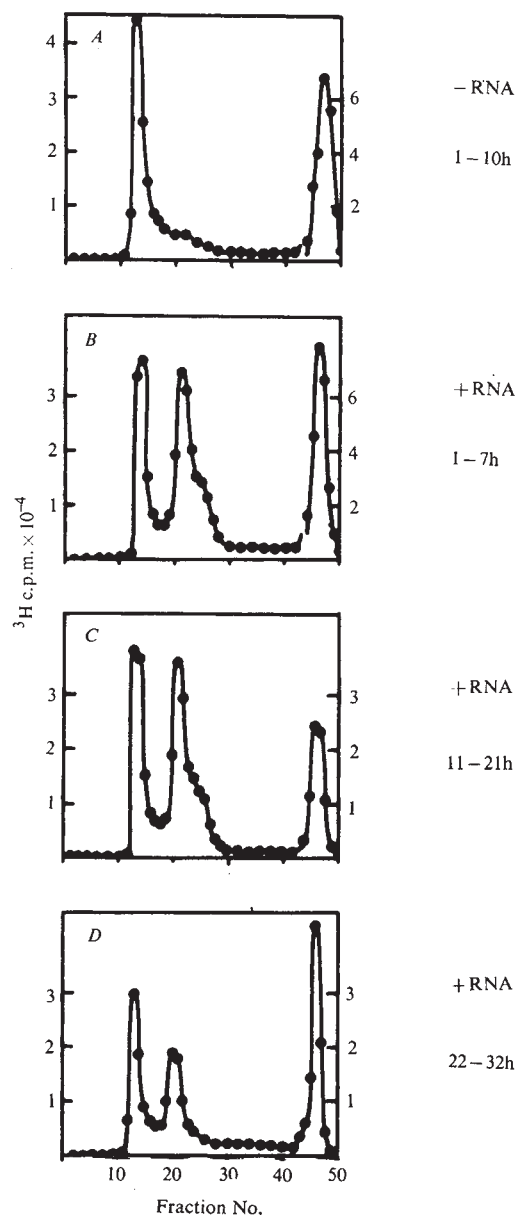


Fig. 3 'Sephadex' analysis of injected oocytes. Each sample of twenty oocytes was injected with haemin, and in the cases of B, C and D with 9S RNA at 400 $\mu\text{g}/\text{ml}$. (about the saturating concentration for these oocytes). Samples were labelled for the periods shown, and frozen at the end of the labelling period; samples C and D were incubated in unlabelled culture medium until the beginning of the labelling period. The radioactivity on the left hand ordinate of each figure refers to the first two 'Sephadex' peaks; that on the right hand ordinate refers to the right hand 'Sephadex' peak of ^3H -histidine.

much smaller, and if negligible would mean that we have overestimated the efficiency of translation by about six times. On the other hand, we have assumed that the same amount of acid-soluble radioactivity as was extracted from oocytes at the end of the labelling period was present throughout the incubation. It is clear from our study of histidine penetration (Fig. 1A) that the cell content of ^3H -histidine increases during the labelling period, and this will have led to our underestimating the efficiency of message translation. Another factor which could have led to a two-fold underestimate of translational efficiency is the leakage of injected mRNA. We know that about half of all injected ^3H -histidine leaks out of oocytes within an hour (Fig. 1A). Finally, it is not certain that every RNA molecule within the 9S preparation injected was a potentially functional message. By assuming that every

molecule within the injected sample is able to serve as a normal Hb message, we can only have underestimated the efficiency of translation.

In Table 4 we compare the efficiency of Hb mRNA translation in different conditions. It is evident that Hb mRNA is translated 100–1,000 times more efficiently in frog oocytes than in a good cell-free system, and if, as seems possible from the above arguments, we have underestimated the efficiency of injected message translation, then the translation of Hb mRNA in frog oocytes compares very favourably with that in normal living reticulocytes.

Can Other mRNAs be Translated?

The results so far reported do not permit the conclusion that every mRNA could be translated in frog oocytes. The successful translation of rabbit 9S RNA in these cells might depend on its possession of one or both of the following properties: (a) it is prepared from a species different from that of the translational system; (b) it codes for a protein never normally synthesized (in detectable amounts) by the host cells.

In the absence of a purified mRNA which codes for proteins normally synthesized in *Xenopus* eggs and oocytes, we have not been able to test the importance of these conditions. But the results of injecting three other types of mRNA into frog eggs and oocytes permit us to conclude that (i) rabbit 9S RNA is not the only heterologous RNA capable of being translated in frog oocytes; (ii) some kinds of artificial polymer cannot be translated, (iii) at least one kind of heterologous mRNA cannot be translated. The three types of mRNA referred to are the mRNA for mouse myeloma protein, AUGpolyU, and f2 bacteriophage virus mRNA.

A 9–13S fraction of RNA extracted from the K-41 mouse myeloma promotes the synthesis of the expected myeloma

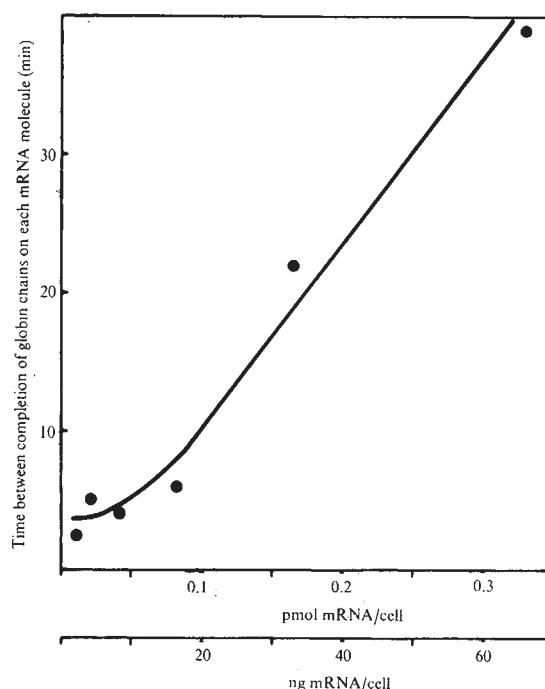


Fig. 4 Each point is based on the 'Sephadex' analysis of twenty oocytes injected with 9S mRNA and haemin, and incubated in medium containing ^3H -histidine for 10 h. The various assumptions made in the calculations in Table 2 are also made here. It is assumed that all molecules in the injected RNA sample were translated with the same efficiency. If it is assumed that there is only one ribosome working on each mRNA molecule at a time, then the ordinate scale shows the time taken to synthesize one globin chain.

light chain immunoglobulins when added to a cell-free system⁷. When this RNA is injected into frog oocytes, 2.5–4% of the proteins labelled by ³H-serine in one experiment, and by ³H-threonine in another experiment, were precipitated by K-41 antiserum; this constitutes a four to six-fold increase above the 0.5% precipitation observed in controls. When the precipitated material was dissociated and passed through a column of 'Sephadex', the experimental but not control samples contained radioactivity which migrated with carrier light chain monomer (Stavnezer, Huang, Gurdon and Lane, in preparation).

Table 3 Efficiency of Hb mRNA Translation in Injected Cells of Unknown Histidine Pool Size

Intracellular concentration of injected mRNA (μg/ml. or ng/cell)*	c.p.m. ³ H-haemoglobin synthesized/cell /h × 10 ⁻³ †	Duration of labelling after injection (h)	No. molecules Hb synthesized/h/mRNA molecule‡
Oocytes			
25	13,340	10	3.4
13	16,940	10	4.8
12.5	8,170	10	4.6
11.8	15,310	10	5.4
11.0	4,250	3	4.8
6.3	5,500	10	4.4
6.3	6,590	10	7.6
6.2	7,050	10	10.4
5.0	15,310	10	5.4
3.12	8,200	10	12.0
1.66	4,690	10	5.8
0.56	4,520	10	8.6
Average of concentrations up to 13 μg/ml.			6.7
Eggs			
20	1,390	2	9.9
10	1,360	2	17.7
5	660	2	14.6
Average			14.1

This table includes the results of five experimental series, carried out on oocytes or eggs of different frogs.

* Calculated from the known concentration of RNA in samples for injection and from the known volume injected into each cell. Leakage, which may occur on a substantial scale, is ignored. 1 egg or oocyte is assumed to contain 1 μl. of solution.

† Based on 'Sephadex G-100' analyses.

‡ The calculations for oocytes assume a pool size of 30 pmol per cell (average of the two values in Table 2), and those for eggs assume a pool size of 91.2 pmol per cell, this being the value obtained from an automatic amino-acid analysis of 350 unfertilized eggs. In other respects calculations were carried out as in Table 2.

The effect of injecting AUGpolyU and ³H-phenylalanine into oocytes has been examined by Woodland and Browne (unpublished). AUGpolyU seems not to stimulate incorporation of ³H-phenylalanine into polyphenylalanine, but it drastically inhibits the synthesis of proteins characteristic of the host cells.

The possibility that bacterial virus mRNA can be translated in frog cells has been examined in collaboration with Dr A. Garen. RNA was prepared from f2 phage by two cold phenol extractions. Immediately after alcohol precipitation it was injected into oocytes at a concentration of about 1 mg/ml. ³H-tyrosine-labelled proteins were extracted from RNA-injected and control eggs and analysed by acrylamide gel electrophoresis. No difference between the two samples was observed, and in neither case was a peak of radioactive protein seen in the region of the gels that contained ¹⁴C-labelled carrier coat protein and replicase. Total protein synthesis was unaffected. Although we have not determined the reason why f2 RNA fails to be translated in frog oocytes, this may be because it requires the translational machinery of bacterial as opposed to animal cells.

Identification of Unknown mRNAs

The opportunity which injected frog cells offer for translating purified mRNA with high efficiency in a normal living cell suggests the application of this system to the identification of unknown mRNAs and their products. The identification of the unknown message for a known protein can be undertaken by separating total RNA into different fractions, injecting these into oocytes or eggs, and assaying for the synthesis of the protein. It is essential to know that the message product is not degraded in oocyte cytoplasm or rendered unrecognizable by complexing with oocyte materials. We have failed to find such effects with several different proteins which had been labelled with ¹²⁵I.

Table 4 Comparative Efficiency of Hb-mRNA Translation

Translational systems	Temperature of incubation (°C)	No. globin chains synthesized per mRNA molecule/h	Half-life of translational system	Total No. translations/half-life of system
Cell-free system (lysate using added mRNA)				
	25*	0.8*	60 min*	0.8*
Rabbit reticulocytes in culture (using endogenous mRNA)				
	37	800†	2–3 h‡ (in culture)	1,840
	20	80†	?	?
Injected frog cells:				
Oocytes (typical)	19	7§	26¶	180
Oocytes (best)	19	24	26¶	625
Eggs	19	14§	?	?

The estimates for the efficiency in frog cells are based on various assumptions, one of which is that the amino-acid pool functional in protein synthesis is the same as the total extractable amino-acid. If this were not so, our estimate would be a maximum of six times too high (see the text for the other assumptions made).

* From ref. 9.

† From ref. 10.

‡ From ref. 11.

§ See Table 3.

|| See Table 2.

¶ See Fig. 3.

If it is required to identify an mRNA and its product when neither have so far been identified, two procedures are available. The first is to look for an increase in the total proportion of intracellular labelled amino-acid that is incorporated into protein. mRNA as pure as our 9S RNA for Hb raises the overall rate of protein synthesis of recipient cells about two-fold when injected at saturating concentrations¹³. The second procedure is to test the ability of a potential message-containing fraction of RNA to compete with a known message (such as Hb mRNA) for a limited supply of the host cells' translational apparatus. Hb mRNA, at a saturating concentration and supplemented by increasing amounts of potential messenger RNA, is injected into a series of test cells; to keep the total RNA injected per cell constant, the increasing amounts of potential mRNA are supplemented with proportionately decreasing amounts of a translationally inert RNA such as 28S rRNA (see below). The existence of mRNA in the potential message fraction is indicated by a reduction in the amount of Hb synthesis when related to acid-soluble radioactivity in the injected cells. Table 5 illustrates the use of this procedure in respect of cultured mouse cell polysomal RNA. Further experiments would be required to prove that the cultured cell RNA reduces Hb synthesis by competition for translational apparatus rather than by some unspecific inhibitory effect; however, this design of experiment can

provide a useful means of screening the potential message content of RNA fractions.

For either of these tests to work satisfactorily it is necessary to know that none of the major classes of cellular RNA (that is, 28S, 18S, 4+5S) with which mRNA is likely to be contaminated will affect Hb mRNA translation. The results in Table 5 and other unpublished experiments have shown that 28S rRNA does not inhibit Hb mRNA translation, and it has been demonstrated elsewhere^{1,2} that neither 28S, 18S nor 4+5S RNA from reticulocytes promotes Hb synthesis when injected on their own. We have found that very high concentrations of RNA, especially of 4+5S RNA, are toxic to some samples of eggs and oocytes; therefore the total amount of injected RNA should, whenever possible, be kept below 50 ng per cell, and the total amount of RNA in all samples to be compared should be kept constant. By attention to these details, we believe that oocytes and eggs can provide a valuable assay system for identifying different kinds of mRNA and their products.

Advantages of Oocyte System

For the study of messenger RNA and its translation, the microinjection of *Xenopus* eggs and oocytes has three special merits. First, the translation of purified mRNA is undertaken in a normal living cell, and is therefore less likely to be affected by artefacts than a cell-free system. Second, injected oocytes are capable of translating mRNA with a high efficiency for long periods. Using our normal labelling procedures, we have obtained, over 24 h, as much as 10⁶ d.p.m. of labelled Hb from 1 ng of injected mRNA. By increasing the amount and range of labelled amino-acids, it would be possible with this system to recognize as little as 1 pg of Hb mRNA. Finally, *Xenopus* oocytes seem to show very little species specificity with respect to the type of mRNA which they can translate. It seems possible that all kinds of eukaryotic and animal virus mRNA may be capable of translation in *Xenopus* oocytes. The large size, resistance to microinjection, and easy availability of *Xenopus* oocytes have relieved us of the incentive to test the translational usefulness of eggs and oocytes from other animal species.

This work was supported by the Medical Research Council. We thank V. A. Moar, C. Gregory, and S. Ayers for technical assistance. G. M. is "Chargé de Recherches" of the Belgian

Table 5 Messenger RNA Competition Experiment

Intracellular concentration of injected RNA (ng/cell)			Protein synthesis as % of acid-soluble c.p.m.	
Hb mRNA	28S rRNA	3T6 RNA	Hb	Endogenous
10	30	0	61.5	111
10	27.5	2.5	35.8	80.1
10	25	5	20.4	35.4
10	17.5	12.5	19.8	45.5
10	10	20	18.4	25.3
10	0	30	12.6	33.2
10	0	0	55.3*	103.1*

The injected cells were large oocytes, which were incubated for 9 h in ³H-histidine before freezing. Analysis was by 'Sephadex'. 3T6 RNA was obtained from polysomes of cultured mouse cells. The variation in values for endogenous protein synthesis is probably due to the variable amounts of uninjected ovarian material in each sample (see text).

*These are average values for three experiments of this kind.

FNRS, and part of his contribution to this work has been made possible by an EMBO fellowship to work in Oxford.

Received May 14, 1971.

- Chantrenne, H., Burny, A., and Marbaix, G., *Prog. Nucleic Acid Res. and Mol. Biol.*, **7**, 173 (1967).
- Gurdon, J. B., *J. Embryol. Exp. Morphol.*, **20**, 401 (1968).
- Gurdon, J. B., *Quart. J. Microsc. Sci.*, **101**, 299 (1960).
- Smith, L. D., and Ecker, R. E., *Current Topics in Devel. Biol.*, **5**, 1 (1970).
- Kutsky, P. B., Eakin, R. M., Berg, W. E., and Kavanau, J. L., *J. Exp. Zool.*, **124**, 263 (1953).
- Ecker, R. E., and Smith, L. D., *Develop. Biol.*, **18**, 232 (1968).
- Stavnezer, J., and Huang, R.-C. C., *Nature*, **230**, 172 (1971).
- Labrie, F., *Nature*, **221**, 1217 (1969).
- Lingrel, J. B., in *Methods in Protein Synthesis* (edit. by Laskin, A. E., and Last, J. A.) (Marcel Dekker, New York, in the press).
- Hunt, T., Hunter, T., and Munro, A., *J. Mol. Biol.*, **43**, 123 (1969).
- Armentrout, S. A., Schinkel, R. D., and Simmons, L. R., *Arch. Biochem. Biophys.*, **112**, 304 (1965).
- Lane, C. D., Marbaix, G., and Gurdon, J. B., *J. Mol. Biol.* (in the press).
- Moar, V. A., Gurdon, J. B., and Marbaix, G., *J. Mol. Biol.* (in the press).

Formation of Antibiotics

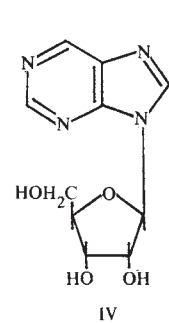
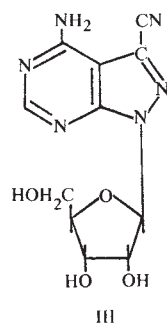
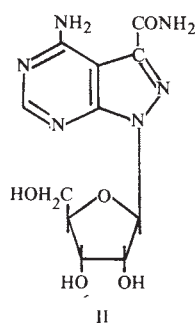
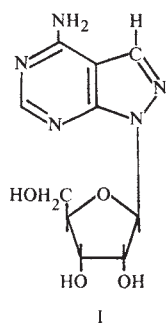
M. M. DHAR & A. W. KHAN

Central Drug Research Institute, Lucknow

Antibiotics could owe their origin to detoxication of a metabolite that is detrimental to the culture producing the antibiotic.

might owe their origin to detoxication of the heterocyclic moieties by peptidation. A consequence of this hypothesis, that introduction of analogues of the heterocycle into the antibiotic synthesizing system should yield analogues of the antibiotic, led to the isolation of quinazoline analogues of the quinoxaline-peptide antibiotic echinomycin both *in vivo*¹ and in a cell-free system². We now suggest that other antibiotics are also microbial metabolites that might owe their origin to detoxication of other metabolites that are detrimental to the culture. It is implied that for its survival, the culture must

It has been suggested¹ that antibiotics, the structures of which consist of heterocyclic moieties attached to peptide sequences,

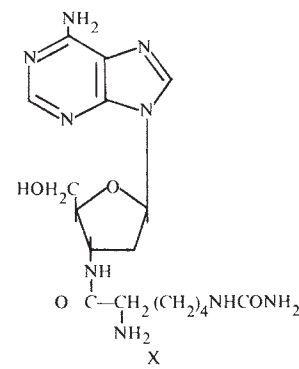
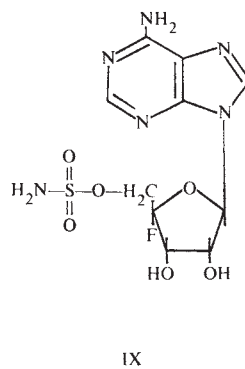
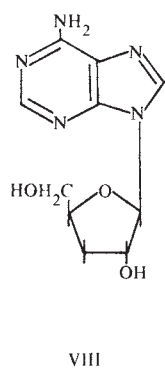
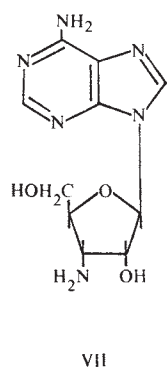
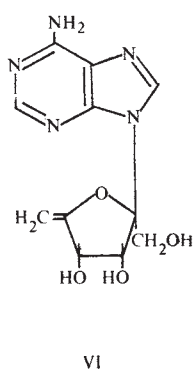
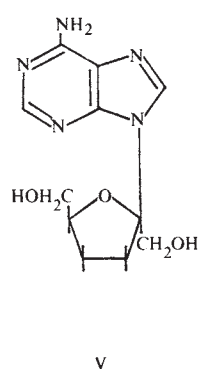


have a mechanism for detoxicating this metabolite or that the metabolite can induce the formation of one or more enzymes required for its detoxication.

Apart from the group of antibiotics that have a peptide sequence attached to a heterocycle, several have structures made up of two types of chemical entity; for example, the nucleosides (heterocycles attached to carbohydrate residues); the macrolides (macrocycles attached to carbohydrate residues); the rifamycins (macrocycle attached to an aromatic system) and the penicillins (carboxylic acid attached to a peptide). We suggest that these antibiotics owe their origin to detoxication of one of the chemical entities by attachment to the other.

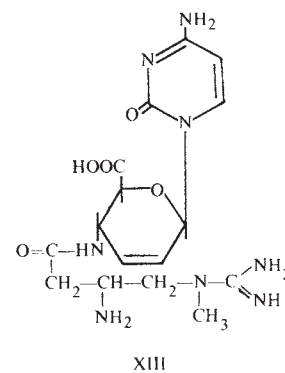
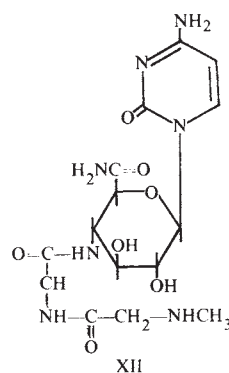
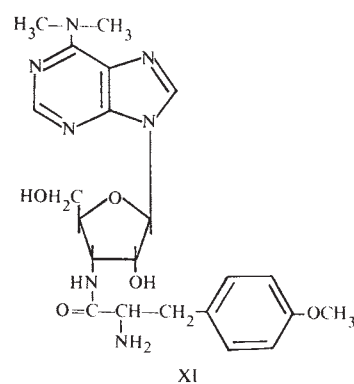
Nucleoside Antibiotics

If nucleoside antibiotics are formed as a consequence of a detoxication process, two types of structure ought to be



encountered. The first, containing unusual heterocycles detoxicated by glycosidation, should contain the usual nucleoside sugars ribose or 2-deoxyribose, and a second, containing unusual sugars, should be glycosides of one of the usual bases: adenine, guanine, cytosine, uracil or thymine. An examination of the structures of known nucleoside antibiotics reveals that they are indeed of these two types.

The antibiotics tubercidin (I), sangivamycin (II), toyocamycin³ (III) and nebularin⁴ (IV) all contain unusual heterocyclic moieties related to adenine. The sugar in every case is D-ribose. On the other hand, psicofuranine (V), decoyinine⁵ (VI), 3'-amino-3-deoxyadenosine⁶ (VII), cordycepin⁷ (VIII), the fluorine containing nucleocidin⁸ (IX) and homocitriculyl-



amino adenosine⁹ (X) are all adenine nucleosides containing unusual sugars.

The related puromycin¹⁰ (XI), however, seems to be a methylated product of a corresponding tyrosyl adenine intermediate.

The pyrimidine antibiotics gougerotin¹¹ (XII) and blastidin^{12,13} (XIII) contain unusual peptidyl aminohexoses, but the base in both cases is cytosine. The other cytosine antibiotic amicitin¹⁴ (XIV) also fits the pattern.

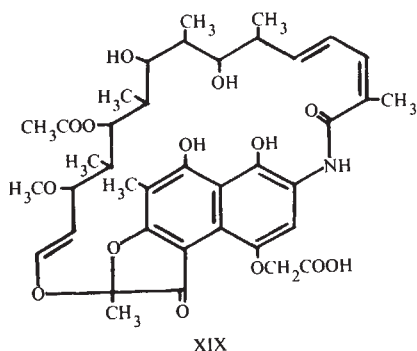
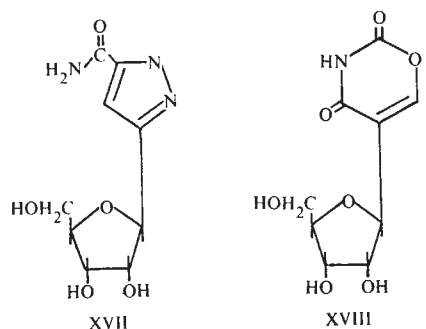
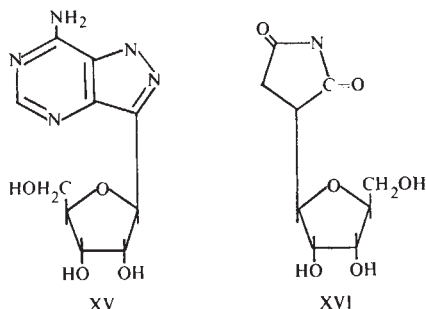
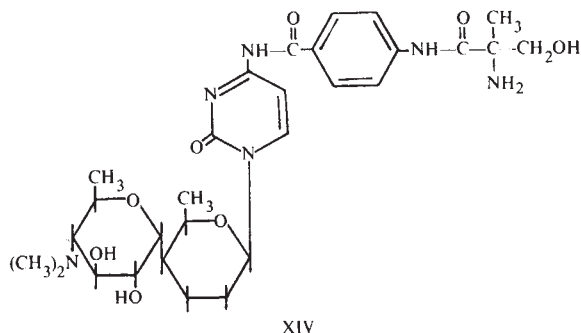
The C-nucleoside antibiotics also fit this pattern. The four known ones: formycin¹⁵ (XV); showdomycin¹⁶ (XVI); pyrazomycin¹⁷ (XVII) and oxazinomycin¹⁸ (XVIII) have unusual aglycones but in every case the sugar residue is ribose.

Rifamycin Group and the Macrolides

An examination of the structure of rifamycin B¹⁹ (XIX) suggests that the molecule is derived from a polyketide chain derived from 2-methylmalonate and a 2-methyl-polyhydroxynaphthalene, which must be derived from mavelonate and a hydroquinone or phenol precursor. The fact that these two parts of the antibiotic molecule are probably derived differently suggests that the naphthalene moiety is detoxicated by attachment to the polyketide derived chain.

More than fifty macrolide antibiotics have been isolated from various actinomycetes and their structures found to possess unique correlations with regard to their stereochemistry²⁰.

It has also been found that the twelve membered lactones are attached to only one sugar residue, a D-4,6-dideoxy or D-6-deoxy pyranose, while the fourteen membered lactones are aglycones of one such sugar as well as a L-2-dideoxypyranose. Sixteen membered lactones are attached to a disaccharide: a L-2,6-dideoxypyranose attached to a D-6-deoxy or D-2,6-dideoxypyranose, which as in the twelve and fourteen membered macrolides is attached to form a β -glycoside of the lactone. In view of the unusual nature of the sugars found in these antibiotics, it seems more likely that the formation of such antibiotics may be initiated by a need to detoxicate one of these sugars, the most common one being desosamine. Studies on the biosynthesis of erythromycin²¹ indicate that the fourteen membered lactone erythronolide B is first attached to the neutral sugar and the mycarosyl erythronolide B converted to erythromycin by formation of a β -glycoside of desosamine and conversion of the mycarose residue to a cladinose.



Corollaries

There are three corollaries to this hypothesis. (1) The toxic metabolite can be used for strain selection. For example,

in the case of the penicillins, it seems that the carboxylic acid is detoxicated by attachment to a 6-aminopenicillanic acid residue. It has been found that only strains which produce penicillin can grow in the presence of significant amounts of phenylacetic acid and that the most efficient penicillin producer can flourish in the highest concentration of phenylacetic acid (P. K. Bhattacharyya, personal communication). Penicillin producers are distinguished by possessing a specific acyl transferase that transfers acyl functions from acyl-coenzyme A to 6-aminopenicillanic acid²². Phenylacetic acid, however, has not been found to induce the formation of this enzyme. (2) The toxic metabolite should in some cases be able to induce the formation of enzymes involved in the synthesis of the antibiotic. (3) The introduction of an analogue of the moiety being detoxicated into the antibiotic synthesizing system, should lead to the formation of an analogue of the antibiotic. This has been demonstrated both *in vivo*^{1,2,3} and in a cell-free system in the case of echinomycin, in the case of the penicillins and recently most elegantly in the case of the bleomycins²⁴.

The hypothesis outlined here is in agreement with two earlier important proposals on the formation of antibiotics^{25,26}, that antibiotics are secondary metabolites formed by normal biogenetic operations; that antibiotics constitute only a small fraction of secondary metabolites that happen to have been found to possess biological activities and that the formation of an antibiotic requires the accumulation of an initiator. It differs from those earlier views in suggesting that the initiators are toxic to their respective cultures and that a large number of antibiotics are from the group of secondary metabolites that are detoxication products of such initiators.

Received June 2; revised July 6, 1971

- ¹ Khan, A. W., Bhaduri, A. P., Gupta, C. M., and Dhar, M. M., *Indian J. Biochem.*, **6**, 220 (1969).
- ² Arif, A. J., Singh, C., Bhaduri, A. P., Gupta, C. M., Khan, A. W., and Dhar, M. M., *Indian J. Biochem.*, **7**, 193 (1970).
- ³ Tolman, R. L., Robins, R. K., and Townsend, L. B., *J. Amer. Chem. Soc.*, **91**, 2102 (1969).
- ⁴ Nakamura, G., *J. Antibiotics*, **14A**, 94 (1961).
- ⁵ McCarthy, J. R., Robins, R. K., and Robins, M. J., *J. Amer. Chem. Soc.*, **90**, 4993 (1968).
- ⁶ Guarino, A. J., and Kredrich, N. M., *Biochim. Biophys. Acta*, **68**, 317 (1963).
- ⁷ Kaczka, E. A., Dulney, E. L., Gitterman, C. D., Woodruff, H. B., and Folkers, K., *Biochem. Biophys. Res. Commun.*, **14**, 452 (1964).
- ⁸ Morton, G. O., Lancaster, J. E., Van Lear, G. E., Fulmer, W., and Meyer, W. E., *J. Amer. Chem. Soc.*, **91**, 1535 (1969).
- ⁹ Kredrich, N. M., and Guarino, A. J., *J. Biol. Chem.*, **236**, 3300 (1961).
- ¹⁰ Baker, B. R., Schaub, R. E., Joseph, J. P., and Williams, J. H., *J. Amer. Chem. Soc.*, **77**, 12 (1955).
- ¹¹ Fox, J. J., Kuwada, Y., Watanabe, K. A., Ueda, T., and Whipple, E. B., *Antimicrobial Agents and Chemotherapy*, 518 (1964).
- ¹² Otake, N., Takeuchi, S., Endo, T., and Yonehara, H., *Tetrahedron Lett.*, 1405 (1965).
- ¹³ Fox, J. J., and Watanabe, K. A., *Tetrahedron Lett.*, 897 (1966).
- ¹⁴ Haskell, T. H., *J. Amer. Chem. Soc.*, **80**, 747 (1958).
- ¹⁵ Koyama, G., Maeda, K., and Umezawa, H., *Tetrahedron Lett.*, 597 (1966).
- ¹⁶ Darnall, K. R., Townsend, L. B., and Robins, R. K., *Proc. US Nat. Acad. Sci.*, **57**, 548 (1967).
- ¹⁷ Streightoff, F. J., Nelson, J. A., Cline, J. C., Gerzon, K., Hoehn, M., Williams, R. H., Gorman, M., and Delong, D. C., *Ninth Conference of Antimicrobial Agents and Chemotherapy*, Abstract No. 18 (Washington DC, 1969).
- ¹⁸ Haneishi, T., Nomura, M., Okazaki, T., Naito, A., Seki, I., Arai, M., Hata, R., and Tamura, C., Meeting of Japanese Antibiotic Research Association, Tokyo, July 1970.
- ¹⁹ Sensi, P., Maggi, N., Furesz, S., and Maffii, G., *Antimicrobial Agents and Chemotherapy*, 699 (1967).
- ²⁰ Celmer, W. D., *J. Amer. Chem. Soc.*, **88**, 5028 (1966).
- ²¹ Martin, J. R., and Rosenbrook, W., *Biochemistry*, **6**, 435 (1967).
- ²² Pruess, D. L., and Johnson, M. J., *J. Bact.*, **94**, 1502 (1967).
- ²³ Yoshida, T., Kumura, Y., and Katagiri, K., *J. Antibiotics*, **21**, 465 (1968).
- ²⁴ Umezawa, H., Antibiotics Symp., Ste Marguerite, Quebec March 1971.
- ²⁵ Bullock, J. D., *Adv. Appl. Microbiol.*, **3**, 293 (1961).
- ²⁶ Woodruff, H. B., *Sixteenth Symp. Soc. Gen. Microbiol.*, 22 (1966).

LETTERS TO NATURE

PHYSICAL SCIENCES

Formation of Glass Spheres on the Moon

I WISH to draw attention to a remarkable morphological similarity between lunar glass spheres and the glass shot which is formed during the manufacture of mineral wool.

Commercial mineral wool is made by pouring a controlled stream of molten slag and silica from a cupola onto a rapidly turning disk, which throws the melt out as a thin horizontal sheet. Just beyond the periphery of the disk the melt encounters an annular steam blast which breaks it up into small droplets and the resultant mixture of approximately equal parts of fibre and shot is discharged into a large chamber where it cools and settles out. The mineral wool mat formed in this way is used in several applications in which its thermal insulation and fire resistant properties are of value. Some of the shot particles remain in the wool but they can be separated in several ways.

Small amounts of a larger diameter shot fraction accumulate in certain positions in the settling chamber and I have measured the size and shape of several hundred of the shot particles from both fractions with a projection microscope, characterizing them by the parameters measured by Mueller and Hinsch¹.

The mineral wool shot, like the lunar spheres, consist chiefly of almost spherical particles, many of which have a "stalk-like appendage"¹, which is the remnant of the attached fibre. Nearly all the forms found in the lunar shot are also present, including "prolate and oblate spheroids, ellipsoids, dumb-bells with and without a central bulge, filaments, and teardrops"¹. Rings are evident only occasionally, but I have not identified "crescents, tops, and spindles" because I am not sure how to distinguish these shapes.

Fig. 1 is a photomicrograph of a typical sample of the mineral wool shot, which may be compared with an example of the Apollo 11 lunar spheres (Fig. 2).

In Table 1 the proportions of the different shapes for both the mineral wool fractions are compared with those found by Mueller and Hinsch for the coarsest fraction of lunar shot. Diameters of the mineral wool shot range from 200 to 800 μm and 100 to 400 μm in the two fractions examined, whereas the lunar shot is from 1 to 500 μm and 70% of the particles are in the 1 to 2 μm range.

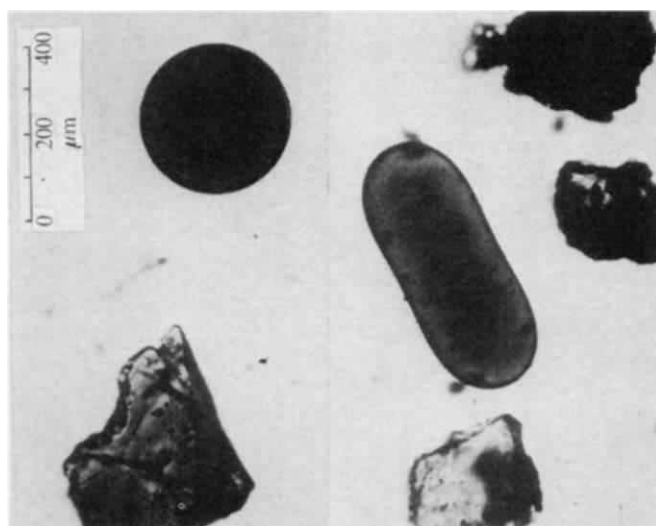


Fig. 2 One spherical and one elongated lunar shot particle (dark brown glass with inclusions). Photomicrograph by Dr D. Stöffler, Tübingen University.

These results establish quite clearly the remarkable overall similarities between these two kinds of glass shot. They are sufficiently striking to suggest strong similarities in their mechanisms of formation. Both may have been formed by the atomization of a mineral melt in a high speed gas stream.

Any one batch of mineral wool shot is of very uniform chemical composition, but there are variations between batches, and between those produced by different manufac-

Table 1 Comparative Morphology of Lunar and Mineral Wool Shot

Sample	Diameter (μm)	No. of spheroids with differing eccentricities			Dumb-bells	Rings	Teardrops
		0 to 10%	10 to 50%	50% +			
Mineral wool, coarse shot	457	57	29	7	6	0	1
Mineral wool, finer shot	276	39	42	7	8	0	4
Lunar shot	350	64	18	11	4	1	2

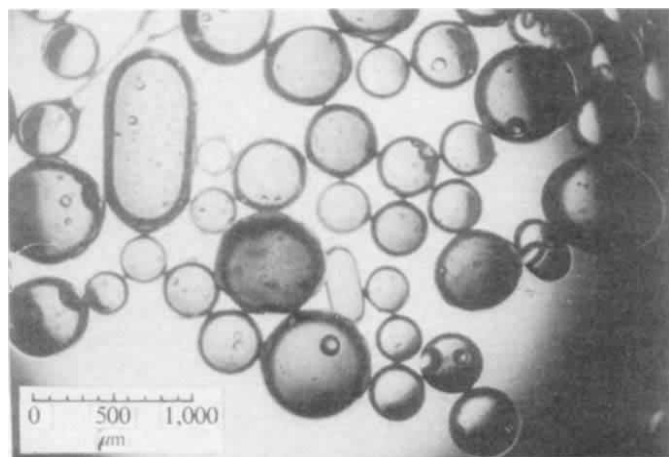


Fig. 1 Typical sample of mineral wool shot showing spheres, ellipsoids and dumb-bell forms (amber to dark brown glass with gas bubble inclusions).

turers, according to the nature of the raw materials used in the melt. The chemical composition of the samples described in this communication is compared in Table 2 with analyses for lunar shot².

Table 2 Composition of Lunar and Mineral Wool Shot

Constituent	Mineral wool shot	Lunar glass spheres of colour indicated				
		Clear	Pale green	Amber	Dark brown	Black
SiO ₂	35.4	49.5	55.4	45.5	41.9	38.1
Al ₂ O ₃	18.3	28.0	1.4	14.0	8.5	4.5
Fe ₂ O ₃ /FeO	0.8	2.5	10.4	12.0	17.4	23.8
CaO	34.9	14.0	20.1	12.0	11.0	7.8
MgO	7.9	2.0	16.7	8.0	6.9	11.9
Na ₂ O	0.9	<0.1	0	<0.1	0.4	0.9

It has been suggested (Mueller, private communication) that the morphology may be influenced by the composition. Although the lunar shot shows marked and highly significant interparticle variation, it is clear that the mineral wool shot contains much more lime than any of the lunar glasses.

The comparison of lunar spheres and mineral wool shot lends additional support to the views put forward by authors such as McKay *et al.*³, who believe that the lunar shot is produced by high speed primary meteorite impacts, presumably as the intensely hot plasma expands through the surrounding solid particles, causing both melting and atomization. The maintenance of geometrical similarity over the whole range from micrometeorites to relatively large impacts explains the approximately constant proportion of elliptical and elongated forms observed over the whole size range by Mueller and Hinsch¹. The smallest particles are formed in very large numbers by the very much more frequent impacts of small meteorites at places where the plasma expands from virtually a point source. Large impacts would produce larger shot because of the less sharply divergent flows.

We may suppose that still larger impacts might produce such large molten particles that they would not have time to solidify before returning to the surface. These "meteoric bombs" would give rise to glassy patches splashed on the surface, or at the centres of small craters if the bomb had sufficient kinetic energy. These glassy patches were explained by Gold⁴ in a different way. More recent work by Anders *et al.*⁵ has, however, shown that they are indeed splashes of foreign molten rock, in which case their identification as secondary impact sites for the molten ejecta from much larger primary impact craters avoids the difficulties encountered in Greenwood's suggestion⁶ that they are themselves primary impact sites.

The fact that mineral wool and lunar shot morphologies are so similar should at least inhibit suggestions that the lunar processes must necessarily have taken place at low ambient pressure and great dilution. Mineral wool is made at one atmosphere in a very intensive process, but the shot so formed does not contain either "grape clusters" or changes in shape obviously attributable to the surrounding gas.

One obvious difference is the absence on the Moon of fine glass fibres. One might suppose that these would be formed in substantial quantity at the same time as the shot. Because of their large surface area and fragile nature it is reasonable to suppose that these fibres would be rapidly degraded in the lunar environment. Their broken fragments should form part of the most finely divided solids fraction in the lunar regolith, and it would seem worth attempting to identify these fragments in the soil.

C. A. CROSS

225 London Road,
Northwich

Received July 27, 1971.

- ¹ Mueller, G., and Hinsch, G. W., *Nature*, **228**, 254 (1970).
- ² Duke, M. B., Woo, C. C., Bird, M. L., Sellers, G. A., and Finkelman, R. B., *Science*, **167**, 648 (1970).
- ³ McKay, D. S., Greenwood, W. R., and Morrison, D. A., *Science*, **167**, 654 (1970).
- ⁴ Gold, T., in *Apollo 11 Preliminary Science Report* (NASA SP 214).
- ⁵ Morgan, J. W., Laul, J. C., Ganapathy, R., and Anders, E., *Science*, **172**, 556 (1971).
- ⁶ Greenwood, W. R., and Heiken, G., *Science*, **168**, 610 (1970).

Eddy Viscosity and the Solar Radiation

DICKE¹, although not the first to do so, has proposed that the deep interior of the Sun rotates faster than the visible exterior (see also Roxburgh², Sakurai³). One result is that the departure of the mass distribution from spherical symmetry would, through its quadrupole moment, cause a contribution to the advance of the perihelion of the planet Mercury, on the basis of Newtonian mechanics, which represents a sufficiently good approximation for this purpose. Unless the fast interior rotation and corresponding interior mass distribution is known and correct allowances made, no precise check of the success of general relativity theory can be made on the basis of the astronomically observed perihelion advance. The main question is whether some such situation concerning the interior rotation can be established, or at least made plausible.

Hide^{4,5,6} has stated that, aside from plausible magnetic complications, an equatorial acceleration in planetary and solar atmospheres cannot be maintained by axisymmetric systems of motion (see also Ward⁷, Starr and Gilman⁸), but requires what actually amounts to a negative eddy viscosity due to nonzonal disturbances in the motion and pressure. I have studied such phenomena observationally for many systems, including laboratory experiments, and their reality cannot be much doubted⁹. The effect is also manifest in numerically integrated models of the Earth's atmosphere (see, for example, Smagorinsky, Manabe and Holloway¹⁰). A requirement is that individual eddies possess a systematic asymmetry usually apparent as a "tilt" with respect to normals drawn across the basic or mean flow, in order to result in the needed covariance of downstream and cross-current velocity components.

Thus, as an illustration, in the instance of the Earth's atmosphere there is a broad maximum of zonally averaged eastward angular velocity around 35°–40° latitude in each hemisphere. Superposed on this mean rotation there are nonzonal eddy components of motion, giving the flow an irregular wavelike appearance at any instant. These eddy components of motion show a special type of asymmetry with respect to the meridians, so that their northward and eastward velocities are instantaneously correlated along the length of individual closed latitude circles at a given height. This correlation is positive so that transport of angular momentum occurs horizontally northward across vertical walls of constant latitude in the vicinity of 30° N, into the zone of maximum mean angular velocity. Around 60° N, on the other hand, the correlation is negative, indicating a tendency (weaker in this case) again to transport angular momentum across latitude walls into the zone of maximum mean rotation. Because the effect is exactly contrary to a normal viscous action—tending to maintain the fast rotation through the horizontal exchange of equal masses of eddying fluid parcels across latitude walls, instead of decreasing it—we may call the process a manifestation of negative eddy viscosity. Some fluid dynamicists prefer to call it pseudo-viscosity. The whole process requires energy sources within the structure of the eddies.

In view of the fact that the several published theoretical treatments of the solar circulation problem like that of Gilman¹¹ and of Durney¹², in addition to the observational studies of Ward^{7,13}, all give evidence of the presence of negative horizontal eddy viscous effects, it is also possible that many stellar

interiors have a fast rotation sustained by a vertical inward angular momentum transport due to eddies (see also Starr and Gaut¹⁴, Starr⁹). If the main action of this kind is located in the convective zone, the shear in mean angular velocity would itself probably induce the proper tilt in the upward and downward convective eddy currents to perpetuate the regime against viscosity effects and against possible Maxwell stresses leading ultimately to joule-heating energy losses. I suggest that theoretical and numerical mathematical models should be constructed, which would allow for this possibility.

V. P. STARR

Department of Meteorology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139.

Received June 7; revised August 4, 1971.

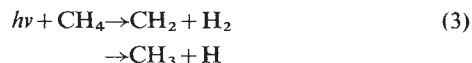
- ¹ Dicke, R. H., *Nature*, **202**, 432 (1964).
- ² Roxburgh, I. W., *Nature*, **213**, 107 (1967).
- ³ Sakurai, T., *Stellar Rotation* (Ohio State Univ., Columbus, Ohio, 1970).
- ⁴ Hide, R., *Planet. Space Sci.*, **14**, 669 (1966).
- ⁵ Hide, R., *J. Atmos. Sci.*, **26**, 841 (1969).
- ⁶ Hide, R., *Nature*, **225**, 254 (1970).
- ⁷ Ward, F., *Pure and Appl. Geoph.*, **58**, 157 (1964).
- ⁸ Starr, V. P., and Gilman, P. A., *Scientific American*, **218**, 100 (1968).
- ⁹ Starr, V. P., *Physics of Negative Viscosity Phenomena* (McGraw-Hill, New York, 1968).
- ¹⁰ Smagorinsky, J., Manabe, S., and Holloway, jun., J. L., *Mon. Weather Rev.*, **93**, 727 (1965).
- ¹¹ Gilman, P. A., *J. Atmos. Sci.*, **24**, 101 (1967).
- ¹² Durney, B., *Astrophys. J.*, **161**, 1115 (1970).
- ¹³ Ward, F., *Astrophys. J.*, **141**, 534 (1965).
- ¹⁴ Starr, V. P., and Gaut, N. E., *Scientific American*, **223**, 72 (1970).

Natural Sources of Atmospheric CO

METHANE is produced at the Earth's surface by various processes, chiefly biological in origin. Koyama¹ estimated an annual source of 3×10^{14} g, with rice paddy fields a major contributor, but did not consider the contribution from natural swamps, and his value for the total source may be low by as much as a factor 3. The data of Bainbridge and Heidt² suggest that they may have observed substantial methane production from the coastal marshes of Louisiana. The important atmospheric sinks for methane are³



and



These reactions constitute permanent destruction of CH_4 . Subsequent chemistry leads to formation of CO and CO_2 , and we shall argue that CO is more likely. The source of atmospheric CO due to CH_4 is comparable with, and may be larger than, that associated with the internal combustion engine.

Reactions (1) and (2) dominate below 80 km. The metastable O atom in (2) is formed by photolysis of ozone and its concentration can be readily determined with available data on reaction rates^{3,4}. Removal of CH_4 in the troposphere is dominated by (1) with OH produced chiefly by

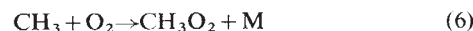


The tropospheric chemistry of OH was discussed recently by Levy⁵ and our chemical model reflects his discussion. (The rate coefficient for (1) was taken from Greiner⁶. For (4) we used relative measurements by Biedenkapp *et al.*⁷ in combination with recent data⁸ for the reaction of $\text{O}(^1\text{D})$ with O_3 .) The reaction



is an important means for recycling odd hydrogen and we adopt Levy's estimate for the rate coefficient, $5 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1}$. Computed altitude profiles for OH and HO_2 and $\text{O}(^1\text{D})$ are illustrated in Fig. 1.

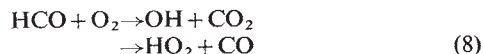
The methyl radical is removed by



and subsequent chemistry leads to production of H_2CO and HCO. The important sink for H_2CO is photolysis⁹,



although loss by rain out may be comparable. Removal of HCO is mainly by



and elementary considerations suggest that the second path, which requires a minimum rearrangement of chemical bonds, should be favoured. The rate for (8) has not been measured, but HCO is also photolysed¹⁰ to yield H and CO, and we estimate the photolysis lifetime to be of the order of 1 h.

A computed altitude profile for CH_4 is illustrated in Fig. 2. We followed procedures described elsewhere⁴ and derived values for the upward flux of CH_4 which are also included in Fig. 2. The computed CH_4 profile is in good agreement with experimental data from a variety of sources. Our calculations imply that reaction (1) is an important sink for CH_4

Fig. 1 Average number densities of OH, HO_2 and $\text{O}(^1\text{D})$ calculated for H_2O densities equal to half the dew point values in the troposphere. The tropopause was taken at 10 km and the stratospheric H_2O content corresponds to a mixing ratio 3×10^{-6} by volume. A profile for ozone appropriate for mid-latitudes was used.

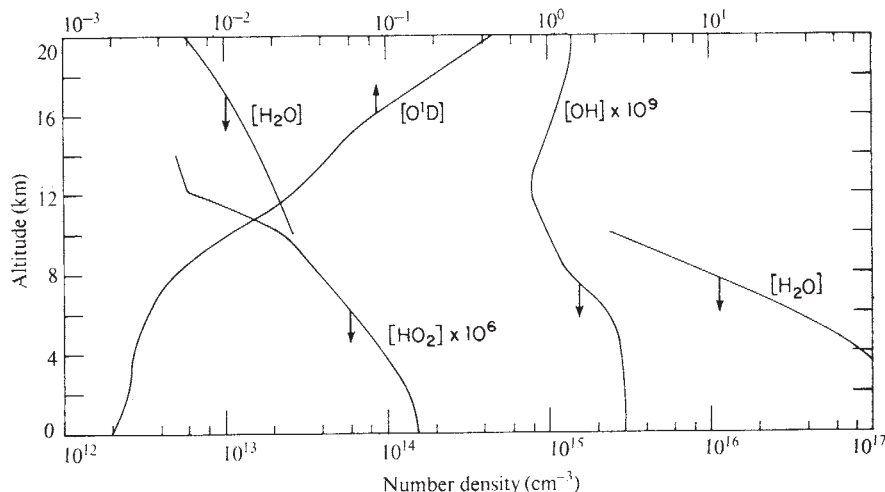
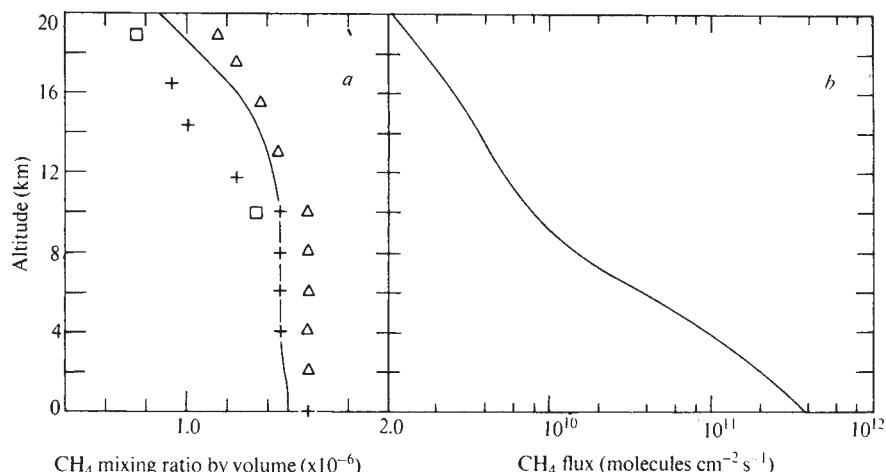


Fig. 2 *a*, Densities of CH₄ calculated by solution of the eddy diffusion equation with diffusion coefficients derived from tracer studies⁴. The solution was constrained to give the observed mixing ratio at the surface and vanishing flux at high altitudes. Measurements by Bainbridge and Heidt² are indicated by Δ (over Texas) and + (over Louisiana). Measurements by Kyle *et al.*¹⁷ increased by a factor 2 to allow for the reduced stratospheric scale height, are indicated by $\square$, globally averaged upward fluxes for CH₄ consistent with densities in *a*. An average flux of 10^{11} molecules cm⁻² s⁻¹ is equivalent to a total flux of 4×10^{14} g yr⁻¹.



and presumably also an important source of CO below 30 km. The need for a significant tropospheric sink for CH₄ was emphasized earlier by Bainbridge and Heidt².

The detailed reaction scheme employed here is somewhat uncertain. A mechanism for recycling HO₂ to OH is provided by reaction (5) but the rate coefficient is not well known. Our choice agrees with Westberg and Cohen⁵ and is consistent with a lower limit (2×10^{-13} cm³ s⁻¹) derived by Niki⁵. A preliminary measurement by Johnston⁵, however, suggests a somewhat smaller value (2×10^{-15} cm³ s⁻¹). Other possibilities for recycling exist, including the reaction of HO₂ with Ozone³ which would be important if the rate coefficient were of order 10^{-14} cm³ s⁻¹. In addition the reaction



followed by photolysis of methylhydroperoxide to yield CH₃O and OH also gives good agreement with the results shown in the figures. It should be noted, however, that even without recycling the OH density at the surface is of order 5×10^5 cm⁻³. The measurements of atmospheric CH₄, in combination with the known source strength, demonstrate that there must be an efficient tropospheric sink for the gas. The ultimate product is likely to be CO and the yield should exceed the anthropogenic source (2×10^{14} g yr⁻¹ or 3×10^{10} molecules cm⁻² s⁻¹).

The model offers ready explanations for a number of puzzling phenomena. It allows one to reconcile diverse estimates for the lifetime of atmospheric CO. Weinstock¹¹, as modified by Junge *et al.*¹², derived a lifetime of 0.2 yr using measurements of ambient atmospheric ¹⁴CO and calculations for the corresponding source strength. Junge *et al.*¹² performed a similar analysis for ¹²CO. Assuming the internal combustion engine to be the major source, they derived a lifetime of 1.3 yr. With the additional source discussed here, CH₄, the lifetime is reduced to 0.3 yr, in harmony with the ¹⁴CO value. Further, using measurements by Greiner¹³ for the reaction



and using the OH profile in Fig. 1, we calculate the absolute value of this lifetime to be 0.3 yr. The predominance of the natural source explains the failure to detect any significant increase in atmospheric CO during the past 20 yr¹⁴, even though production by internal combustion engines has increased by about a factor 2. It further accounts for the similarity of CO mixing ratios observed in the high tropospheres of both northern and southern hemispheres¹². Some asymmetry would be expected if the car were the dominant source, because its contribution should be concentrated in the northern hemisphere and also because the time constant for interhemispheric exchange is about 5 yr¹⁴. Measurements in the high troposphere are especially useful in that the influence of local surface conditions, which can act either as sources or sinks of CO, are minimized.

Our model is supported also by measurements of formalde-

hyde in rain¹⁵. This gas exhibits the same T/H ratio as atmospheric methane¹⁵. The rain measurements imply a gas phase mixing ratio, H₂CO to air, of 1.3×10^{-9} by volume at 5° C¹⁶. The corresponding photolysis rate to yield CO is 5×10^{11} molecules cm⁻² s⁻¹ if we assume a mixed distribution of the H₂CO and adopt mean isolation conditions. Our model predicts a total CO production of 3.7×10^{11} cm⁻² s⁻¹, in satisfactory agreement with this result.

The internal combustion engine may locally determine the atmospheric CO abundance. There is strong evidence, however, that the global level is governed by natural sources and oxidation of methane is likely to be a major contributor.

J. C. MCCONNELL
M. B. McELROY

Center for Earth and Planetary Physics,
Harvard University

S. C. WOFSY

Department of Chemistry,
Harvard University

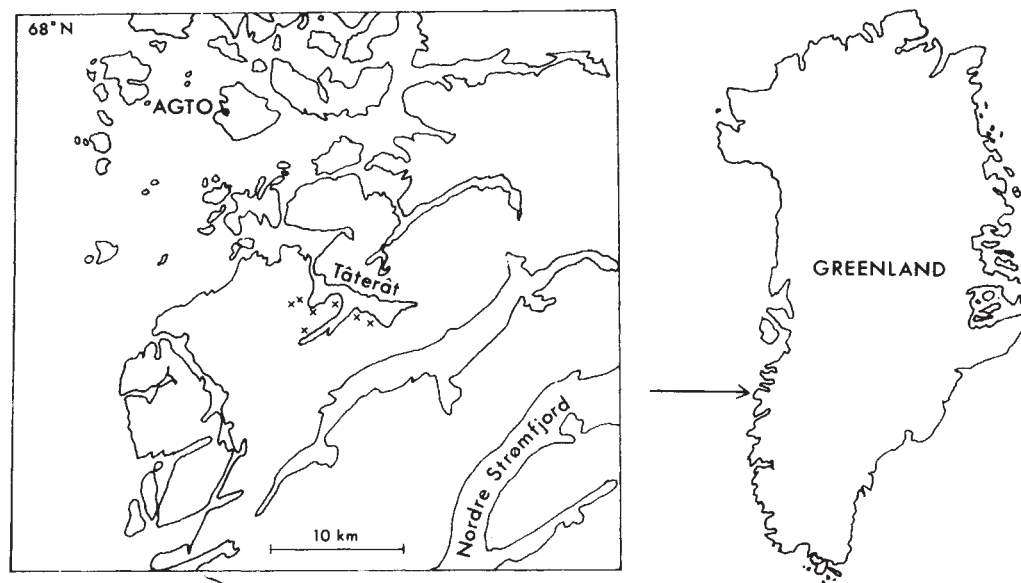
Received July 28, 1971.

- ¹ Koyama, T., *J. Geophys. Res.*, **68**, 3971 (1963).
- ² Bainbridge, A. E., and Heidt, L. E., *Tellus*, **18**, 221 (1966).
- ³ Nicolet, M., *Ann. Geophys.*, **26**, 531 (1970).
- ⁴ McElroy, M. B., and McConnell, J. C., *J. Atmos. Sci.* (in the press).
- ⁵ Levy, II, H., *Science*, **173**, 141 (1971).
- ⁶ Greiner, N. R., *J. Chem. Phys.*, **53**, 1070 (1970).
- ⁷ Biedenkapp, D., Hartshorn, L. G., and Bair, E. J., *Chem. Phys. Lett.*, **5**, 379 (1970).
- ⁸ Gauthier, M., and Snelling, D. R., *J. Chem. Phys.*, **54**, 4317 (1971).
- ⁹ McQuigg, R. C., and Calvert, J. G., *J. Amer. Chem. Soc.*, **91**, 159 (1969).
- ¹⁰ Johns, J. W. C., Priddle, S. H., and Ramsay, D. A., *Diss. Faraday Soc.*, **35**, 90 (1963).
- ¹¹ Weinstock, B., *Science*, **166**, 224 (1969).
- ¹² Junge, C., Seiler, W., and Warneck, P., *J. Geophys. Res.*, **75**, 2217 (1971).
- ¹³ Greiner, N. R., *J. Chem. Phys.*, **51**, 5049 (1969).
- ¹⁴ Pressman, J., and Warneck, P., *J. Atmos. Sci.*, **27**, 155 (1970).
- ¹⁵ Shearer, E. C., *Relationships among Atmospheric Formaldehyde, Methane and Krypton*, thesis, Univ. Arkansas (1969).
- ¹⁶ Credali, L., Mortillaro, L., Galizzo, G., Russo, M., and Checchi, C. de., *Chim. Ind. (Milan)*, **47**, 732 (1965).
- ¹⁷ Kyle, T. G., Murcray, D. G., Murcray, F. H., and Williams, W. J., *J. Geophys. Res.*, **74**, 3421 (1969).

Early Precambrian Impact Structure and Associated Hyalomylonites near Agto, West Greenland

WORK on the Isortoq granulite facies rocks of the Nagsugtôqidian mobile belt¹ in West Greenland has revealed an area where the rocks show features believed to be caused by shock metamorphism, while in neighbouring areas small

Fig. 1 Crosses indicate locations of shock-metamorphic rock-types.



veins of glass-mylonite (hyalomylonite) have been generated in pre-existing regional thrust zones.

The most convincing evidence of shock metamorphism was found about 20 km south-east of Agto along the western branches of the Tåteråt fjord. Although no features suggesting an impact structure can be seen on topographic maps, the enderbitic gneisses at this locality are more or less texturally destroyed and locally more than half of the rocks consist of a very irregular pattern of small black veins embracing gneiss fragments—"pseudotachylytes". The ground mass in these veins is black and aphanitic, and decorated and non-decorated planar elements² are seen in quartz, plagioclase and K-feldspar both in the gneiss and in the fragments in the pseudotachylyte.

Apart from diaplectic glasses³ of the above mentioned minerals every transition from diaplectic to normal fused glass seems to be present. Felsic grains in the pseudotachylyte as well as in the surrounding gneiss locally have a rim of fused material shown by gently twisting colourless flow lines in their border zones.

Pyroxenes and amphiboles are filled with additional planar elements, deformation lamellae and deformation twins. The pleochroism is no longer present and the birefringence is reduced to low values². Locally the mafics are destroyed and only black-brown isotropic areas (oxidized pyroxene and amphibole⁴) are present. In the garnets up to four systems of very regular planar features are observed. Apatite also has planar features.

The texture of the gneiss varies between normal hemigranoblastic (with planar elements) to almost completely destroyed mortar-like textures.

Strong shock metamorphic effects were observed within an area of only a few square kilometres but effects have been found locally within an area of at least 30 km².

A hyalomylonite occurs in a thrust zone about 1.5 km from the area showing the most intense shock metamorphism. The thrust zone can be followed over a distance of roughly 130 km from the Inland Ice to the Davis Strait and belongs to an extensive system of fault and thrust zones in West Greenland⁵, probably of pre-Nagssugtoqidian initiation. Different types of normal crush-rocks are found in these zones.

The hyalomylonite vein which occurs in a conformably enclosed relic gneiss lens in the thrust zone can be followed about 10 m and measures a maximum 5 cm across. This flat ellipsoidal vein has knife-sharp margins and exactly the same orientation as the thrust zone and the foliation in the gneiss lens. The vein consists of about 85% homogeneous glass and about 15% fragments, which, except for one zircon grain, are entirely of quartz and feldspar, often with planar

elements. The glass is in contact with the gneiss, which is highly crushed with planar elements and the mafics locally in an oxidized state.

The glass contains many spherulites a few microns in diameter with dark nuclei. Flow lines are only seen in the glass around the felsic inclusions. Minute, secondary calcite-filled cracks are observed in the glass.

Chemical analysis of the hyalomylonite and the surrounding gneiss both for major and trace elements shows a very close similarity between the two. The refractive index of the glass is 1.530 ± 0.001 .

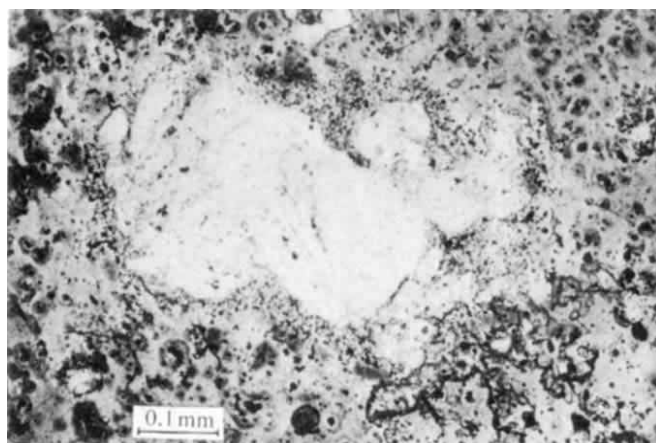


Fig. 2 Felsic grain enclosed in glass, viewed with plane-polarized light.

A K/Ar determination carried out on the glass gave an age of $3,070 \pm 70$ m.y.⁶. Only a very weak devitrification is observed locally, probably because of the dry conditions in the Isortoq complex as a whole⁷, which would allow only thermal reconstruction, a very slow process, to occur⁸.

The generally accepted age of the Nagssugtoqidian mobile belt is 1,790–1,650 m.y.⁹, but several authors^{5,10} believe that pre-Nagssugtoqidian rocks and structures are common in the area mentioned.

There is also a possibility that excess Ar is present, but because glasses are generally believed to be only weakly retentive to Ar and usually give low ages¹¹, further work is necessary to clarify the meaning of this unexpectedly old age.

The formation of the hyalomylonite vein is considered to be due to instantaneous movements in cold and rigid surroundings.

About 6 km from the most shocked area other hyalomylonites are found. Here the glass is enclosed in a mylonitic rim in contact with the gneiss, which is also severely crushed. Different types of recrystallization, partly devitrification and partly crystallization from a melt, are observed.

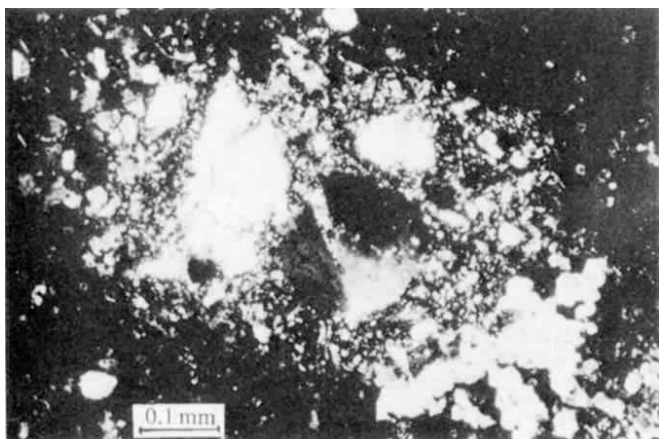


Fig. 3 Same as Fig. 2, but viewed through crossed nicols.

Different crush-rocks from tectonics are present in the Agto area, but the features discussed here are only seen in the vicinity of the shock metamorphic locality. Metamorphism giving rise to such features seems possible only as a result of meteorite impact (astroblesmes¹²) and the observations around Tåterât seem indicative of an impact in early Pre-Cambrian time.

Detailed mineralogical investigations which have been carried out on the shocked minerals and hyalomylonites will be presented later.

The work was carried out under the auspices of the Geological Survey of Greenland (GGU) and Institute of General Geology, Copenhagen. Dr C. K. Brooks assisted with the preparation of the manuscript.

VAGN JENSEN

Institut for Almen Geologi,
Østervoldgade 5-7,
1350 Copenhagen K

Received March 12; revised July 30, 1971.

- ¹ Noe-Nygaard, A., *Rep. Eighteenth Intern. Geol. Cong. GB*, Part 13, 199 (1948).
- ² v. Engelhardt, W., Stöffler, D., and Schneider, W., *Geologica Bavarica*, No. 61, 229 (1969).
- ³ v. Engelhardt, W., and Stöffler, D., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 159 (Mono Book Corp., 1968).
- ⁴ Chao, E. C. T., in *Shock Metamorphism of Natural Materials* (edit. by French, B. M., and Short, N. M.), 135 (Mono Book Corp., 1968).
- ⁵ Escher, A., *Rapp. Grønlands Geol. Unders.*, No. 11, 24 (1966).
- ⁶ Larsen, O., *Rapp. Grønlands Geol. Unders.*, No. 19, 62 (1969).
- ⁷ Ramberg, H., *Medr. Dansk. Geol. Foren.*, 12, 27 (1951).
- ⁸ Marshall, R. R., *Bull. Geol. Soc. Amer.*, 72, 1493 (1961).
- ⁹ Pulvertaft, T. C. R., *Rep. Twenty-third Intern. Geol. Congr. Czechoslovakia*, Sect. 4, 89 (1968).
- ¹⁰ Sørensen, K., *Rapp. Grønlands Geol. Unders.*, No. 27 (1970).
- ¹¹ Webb, A. W., and McDougall, I., *Earth Planet. Sci. Lett.*, 3, 41 (1967).
- ¹² Dietz, R., *S. Sci. Amer.*, 205, 50 (1961).

BIOLOGICAL SCIENCES

Quinacrine Staining of Chromosomes and Evolutionary Studies in *Drosophila*

PARTICULAR chromosome regions have been found to fluoresce intensely after staining with quinacrine and related compounds¹⁻¹⁶, and we have been studying the pattern of distribution of these regions in the chromosomes of various species and laboratory stocks from the genus *Drosophila*. We find that these patterns vary in interesting ways and that they are a characteristic of chromosomes that can be used in studies of evolutionary cytogenetics in much the same way as structural rearrangements¹⁷⁻¹⁹. The following are among our observations.

(1) *Drosophila melanogaster* differs consistently from its sibling species *D. simulans* in the pattern of the brightly fluorescent regions. The two species can be distinguished by the patterns of bright fluorescence of either the mitotic chromosomes of the neuroblasts or the polytene chromosomes of the salivary gland cells. In neuroblast metaphases of *D. melanogaster*, every chromosome has brightly fluorescing regions. In *D. simulans*, such regions are found on only the Y and fourth chromosomes of metaphase cells. In salivary gland cells of *D. melanogaster* (Oregon-R) there is always an intensely fluorescent region near the base of chromosome IIIIR at approximately 81F; and, in some individuals, there is also a second brightly fluorescent region more distally at approximately 83D (see (5) below). *D. simulans* salivary gland cells have no brightly fluorescent regions on the third chromosome. In *D. melanogaster* (Oregon-R) there are two intensely fluorescent regions on chromosome IV: one near the base in region 101 and one in the middle of IVR at approximately 102D. In *D. simulans* salivary gland cells there is always a brightly fluorescent region at the base of the fourth chromosome near the chromocentre; there may be a second intensely fluorescent region at the tip of chromosome IV depending on the stock (see (2)). Dots of brightly fluorescent material not obviously associated with any chromosome are frequent within the chromocentre of both species. These observations suggest the usefulness of quinacrine staining for analysing genetic and evolutionary relationships among sibling species²⁰⁻²³.

(2) Certain laboratory stocks of *D. simulans* differ with regard to the intensely fluorescent regions of the fourth chromosome. In individuals from three laboratory stocks (wild type stocks derived from populations in Honduras and Madison and a *vermillion* stock from the California Institute of Technology) such regions are found at both the base of the fourth chromosome adjacent to the chromocentre and at its tip. These two regions usually pair ectopically, and the whole chromosome then takes on a U shape. In cells from larvae of the fourth stock (*yellow, white* from the California Institute of Technology) only the base of the fourth chromosome fluoresces brightly after quinacrine staining. In this stock there is no noticeable ectopic pairing of the base with the tip of the chromosome, and the fourth chromosome is straight²⁴. Because *D. simulans* is one of the three widespread species of *Drosophila* considered to be monomorphic with regard to chromosome structure²⁵, our observations indicate that quinacrine staining can reveal chromosome polymorphisms among and within populations that contain no gross rearrangements and are therefore classified as chromosomally monomorphic. All four stocks are classifiable as *D. simulans* on the basis of their male genitalia²⁶, and breeding experiments indicate no genetic isolation among them.

(3) A survey of a few of the principal species groups within the genus reveals that not all species have chromosome regions which fluoresce brightly after quinacrine staining and that the distribution of such species follows generally what is known of the taxonomic relations within the genus. Thus, *D. melanogaster*, *D. simulans* and *D. virilis* have fluorescent bands, while

D. hydei, *D. melanopalpa*, *D. repleta*, *D. peninsularis* and *D. tumiditarsus* do not. It will be interesting to correlate such cytological observations with information about the composition of DNA, especially satellite DNA, of the various species²⁷.

(4) A band that fluoresces intensely after quinacrine staining is found on the dot chromosome of the Hawaiian species, *D. hemipeza* but not in *D. grimshawi*, another Hawaiian species.

These observations open the possibility of comparing closely related species, including homosequential or nearly homosequential ones, found on these islands. Homosequential species²⁸⁻²⁹ are morphologically distinct, reproductively isolated species with identical banding patterns along their polytene chromosomes. Differences in the fluorescence patterns of homologous loci may therefore indicate alleles which differ to an evolutionarily significant degree.

(5) In some individuals of one laboratory stock of *D. melanogaster* (Oregon-R) one of the salivary gland chromosome regions (approximately 83D) fluoresces brightly after quinacrine staining; in others it does not. In still other individuals only a small amount of brightly fluorescent material is seen at this region. The analysable salivary gland cells from any one larva are all the same in this regard. It is not clear that the gene or genes responsible for bright fluorescence after staining with quinacrine are located at those chromosome regions where it is observed. To classify the phenomenon as a polymorphism, we should therefore like to be able to demonstrate heterozygous individuals within the population. In several different salivary gland preparations from this stock we have observed a few cells in which there is asynapsis for an appreciable length of chromosome IIIR, including region 83D. In such cells large portions of each homologue of IIIR can be examined separately. In some individuals we have found cells that fluoresce brightly at region 83D in one homologue but not in the other. In other individuals we have found cells that have bright fluorescence at region 83D on each of the asynapsed homologues. In still others we have found cells that show no bright fluorescence at this region in either asynapsed homologue. There must therefore be some individuals in this population which are heterozygous for bright fluorescence at region 83D, and they are probably those with a small amount of brightly fluorescent material at this region.

Taken together, these findings, which we shall report in detail later^{30,31}, indicate that the property of fluorescing brightly after staining with quinacrine is a phenotypic character observable at the level of specific chromosome regions in evolutionarily interesting situations. Chromosome differences have until now been observable within and among populations chiefly on the basis of gross structural rearrangements which *de facto* involve changes in gene order. The quinacrine technique has the advantage of being able to reveal chromosome differences within and among populations in the absence of gross changes in gene order. We suggest that this technique is promising as a tool in the analysis of chromosome differences between, and chromosome polymorphisms within, both natural and experimental populations of *Drosophila* and quite possibly other plant and animal taxa as well.

We thank Dr Lynn H. Throckmorton for confirming our classification of all *D. melanogaster* and *D. simulans* stocks used in this study, and Dr Harvey A. Bender and Dr Ronald S. Ostrowski for confirming our identification of salivary gland chromosome positions. Dr Barr is the recipient of a US National Institutes of Health Research Career Development Award; Dr Ellison is a US National Science Foundation post-doctoral fellow. This work is supported by a grant from the American Cancer Society and by funds administered by the Graduate and Medical Schools of this university.

H. J. BARR
J. R. ELLISON

Department of Anatomy,
University of Wisconsin,
Madison, Wisconsin 53706

Received April 15, 1971.

- ¹ Caspersson, T., Farber, S., Foley, G. E., Kudynowsky, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., *Exp. Cell. Res.*, **49**, 219 (1968).
- ² Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell. Res.*, **58**, 128 (1969).
- ³ Caspersson, T., Zech, L., Modest, E. J., Foley, G. E., Wagh, U., and Simonsson, E., *Exp. Cell. Res.*, **58**, 141 (1969).
- ⁴ Zech, L., *Exp. Cell. Res.*, **58**, 463 (1969).
- ⁵ Barlow, P., and Vosa, C. G., *Nature*, **226**, 961 (1970).
- ⁶ Caspersson, T., Zech, L., and Johansson, C., *Exp. Cell. Res.*, **62**, 490 (1970).
- ⁷ Caspersson, T., Zech, L., and Johansson, C., *Exp. Cell. Res.*, **60**, 315 (1970).
- ⁸ Caspersson, T., Zech, L., Johansson, C., and Modest, E. J., *Chromosoma*, **30**, 215 (1970).
- ⁹ George, K. P., *Nature*, **226**, 80 (1970).
- ¹⁰ Pearson, P. L., and Bobrow, M., *Nature*, **226**, 959 (1970).
- ¹¹ Pearson, P. L., Bobrow, M., and Vosa, C. G., *Nature*, **226**, 78 (1970).
- ¹² Vosa, C. G., *Chromosoma*, **30**, 366 (1970).
- ¹³ Vosa, C. G., *Chromosoma*, **31**, 446 (1970).
- ¹⁴ Borgaonkar, D. S., and Hollander, D. H., *Nature*, **230**, 52 (1971).
- ¹⁵ Shettles, L. B., *Nature*, **230**, 52 (1971).
- ¹⁶ Sumner, A. T., Robinson, J. A., and Evans, H. J., *Nature New Biology*, **229**, 231 (1971).
- ¹⁷ Patterson, J. T., and Stone, W. S., *Evolution in the Genus Drosophila* (Macmillan, New York, 1952).
- ¹⁸ Ford, E. B., *Genetic Polymorphism* (Faber and Faber, London, 1965).
- ¹⁹ Dobzhansky, T., *Genetics and the Evolutionary Process* (Columbia University Press, New York, 1970).
- ²⁰ Hubby, J. L., and Throckmorton, L. H., *Amer. Naturalist*, **102**, 193 (1968).
- ²¹ O'Brien, S. J., and MacIntyre, R., *Amer. Naturalist*, **103**, 97 (1969).
- ²² Ayala, F. J., Mourão, C. A., Pérez-Salas, S., Richmond, R., and Dobzhansky, T., *Proc. US Nat. Acad. Sci.*, **67**, 225 (1970).
- ²³ Entingh, T. D., *Genetics*, **66**, 55 (1970).
- ²⁴ Horton, I. H., *Genetics*, **24**, 234 (1939).
- ²⁵ Carson, H. L., in *The Genetics of Colonizing Species* (edit. by Baker, H. G., and Stebbins, G. L.), 503 (Academic Press, New York, 1965).
- ²⁶ Sturtevant, A. H., *Genetics*, **5**, 488 (1920).
- ²⁷ Hennig, W., Hennig, I., and Stein, H., *Chromosoma*, **32**, 31 (1970).
- ²⁸ Carson, H. L., Clayton, F. E., and Stalker, H. D., *Proc. US Nat. Acad. Sci.*, **57**, 1280 (1967).
- ²⁹ Carson, H. L., Hardy, D. E., Spieth, H. T., and Stone, W. S., in *Essays in Evolution and Genetics* (edit. by Hecht, M. K., and Steere, W. C.), 437 (Appleton-Century-Crofts, New York, 1970).
- ³⁰ Ellison, J. R., and Barr, H. J., *Chromosoma* (in the press).
- ³¹ Ellison, J. R., and Barr, H. J., *Genetics* (in the press).

Chromatid Disjunction in Unfertilized Ageing Oocytes

AGEING mammalian oocytes, which have spent longer than the optimum time in the oviduct without being fertilized, may, after fertilization, give rise to abnormal embryos in which aneuploidy is frequent¹. We have now observed a change in the organization of the chromosomes of ageing secondary oocytes of mice, which may account for the occurrence of aneuploidy at the second meiotic division.

As in most mammals, the first meiotic division is completed in the ovary, and the ovulated oocytes are propelled along the oviduct in a state of arrested meiosis at second metaphase until—and if—they are fertilized. In the second meiotic division, which is activated by sperm entry at fertilization, the chromatid pairs which constitute each chromosome of the haploid oocyte disjoin and move towards opposite poles, one of which is destined to become the second polar body. We have evidence that the disjunction of chromatid pairs sometimes occurs before fertilization, but after prolonged sojourn in the oviduct. Subsequent fertilization of an oocyte in such circumstances may lead to a viable zygote in which, however, the synchrony of the partitioning of the chromosomal elements is so disturbed that aneuploidy is more probable.

When we induced virgin mice, 5–8 weeks old, to superovulate by the administration of hormones, we noted after they had been killed, that the contents of their oviducts were variable, particularly with respect to cohesiveness and location of the cumulus-oocyte mass. The occurrence of premature chromatid disjunction, observed in the meiotic chromosome preparations² was more frequent when the ductal contents were characterized by dispersed cumulus, location of most of the oocytes in the subampullar region and the presence of some oocytes devoid of follicle cells (Table 1). Those conditions may be more accurately indicative of “ageing” oocytes than the lapse of time after administration of the ovulatory hormone because (1) the effects of exogenous hormones are superimposed on the endogenous hormonal state, variable in mature female mice, and (2) although the precise role of the cumulus mass in which the oocytes are ovulated has not been defined, it is known that the cumulus is dispersed and the coronae of follicle cells fall away as the oocytes are propelled along the oviduct³.

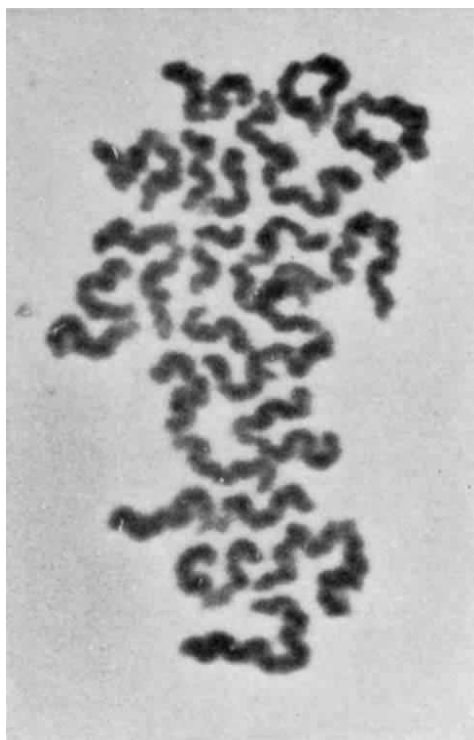


Fig. 1 Chromosome complement of secondary oocyte at second metaphase. All chromatid pairs are joined at their centromeres.

The relationship between anomalous development and experimentally induced delay in fertilization is well established¹. Our observations, consonant with those of another investigator⁴ who described morphological changes in the spindle assembly of mouse oocytes that had a prolonged stay in the oviduct, provide a cytologic basis for attributing those developmental abnormalities to impairment of the machinery of the second meiotic division. More important, they present a mechanism whereby the increased frequency of trisomy in offspring of older women may, as proposed, be due to the greater probability of delayed fertilizations.

It is significant that the type of chromosome alteration described here is not seen in the pathway of degeneration of the first polar body. Even in an advanced state of degradation, the polar body chromosomes remain as chromatid pairs, joined at their centromeres². The disjunction of sister chromatids in unfertilized secondary oocytes appears, therefore, to be a characteristic of intraductal ageing rather than a non-specific type of change in degenerating meiotic chromosomes.

Table 1 Occurrence of Chromatid Disjunction in Unfertilized Secondary Oocytes in Relation to the State of the Intraductal Contents

Fresh cumulus *			Aged cumulus †		
Oviduct	No. of oocytes ‡	No. with disjoined chromatids	Oviduct	No. of oocytes ‡	No. with disjoined chromatids
2a	15	2	1a	12	3
3a	8	0	1b	2	1
3b	9	0	2b	9	2
5b	8	0	4a	7	2
7a	8	0	4b	2	1
7b	8	0	5b	12	2
10a	7	0	6a	15	1
10b	4	0	6b	7	1
11a	11	0	8a	10	2
11b	2	0	8b	9	1
12a	11	0	9a	8	0
12b	1	0	9b	0	0
15a	5	0	13a	4	0
15b	5	0	13b	0	0
16a	7	0	14a	4	0
16b	2	0	14b	4	0
17a	8	0	18a	6	0
17b	7	0	18b	1	0
20a	9	0	19a	1	0
20b	8	0	19b	0	0
21a	9	1	22a	8	2
21b	0	0	22b	2	0
24a	6	0	23a	6	2
24b	6	0	23b	2	0
24	164	3	24	131	20

* Oocyte-cumulus mass was cohesive, located in the ampullar region of the duct, and there were no separate oocytes, free of follicle cells. Chromatid disjunction was observed in 1.8% of the chromosome preparations.

† Cumulus mass fell apart in the saline solution in which the oviduct was sectioned, most of the oocytes were located in the subampullar region, and some oocytes, free of follicle cells, were present. Chromosome disjunction was observed in 15% of the preparations.

‡ Number of chromosome preparations in which the centromeres could be seen as either intact or divided in at least ten chromatid pairs.

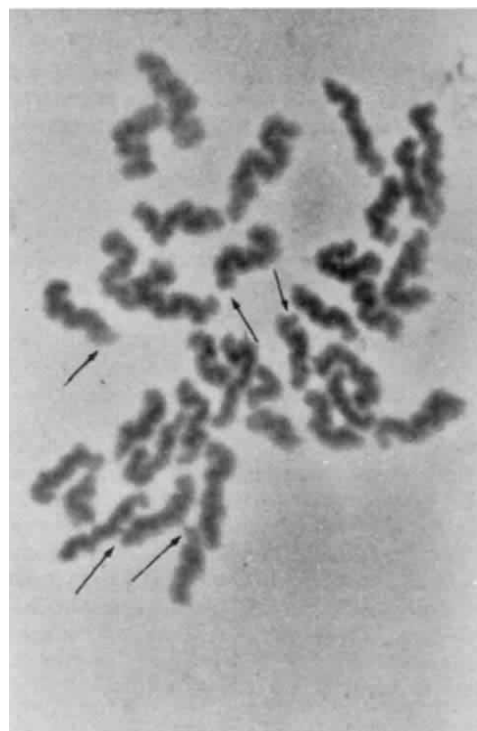


Fig. 2 Chromosome complement of secondary oocyte obtained from oviduct with “aged cumulus” (Table 1). Some sister chromatids have disjoined (arrows); others are still joined.

This work was supported by a grant from the Damon Runyan Memorial Fund Inc.

TOBY C. RODMAN

Department of Anatomy,
Cornell University Medical College,
1300 York Avenue,
New York,
New York 10021

Received April 2; revised June 7, 1971.

¹ Austin, C. R., *J. Reprod. Fert.*, Suppl. 12, 39 (1970).

² Rodman, T. C., *Exp. Cell Res.*, 66 (1971).

³ Blandeau, R. J., in *Progress in Gynecology* (edit. by Sturgis, S. H., and Taylor, M. L.), 5, 58 (Grune and Stratton, New York, 1970).

⁴ Szollosi, D., *Amer. J. Anat.*, 130, 209 (1971).

⁵ German, J., *Nature*, 217, 516 (1968).

Sequential Activation of Clotting Factors during Blood Coagulation

THE existence of activated forms of blood clotting factors was suggested more than twenty years ago. Ware and Seegers¹, pointing out a difference between the plasma and the so-called serum factor V, postulated a partial modification of the clotting factor by thrombin, which resulted in more potent activity. After activation, procoagulant action rapidly declines². Rapaport³ described the activation of the anti-haemophilic factor VIII by traces of thrombin. Thus, it is likely that the first traces of thrombin, produced during coagulation, convert factors V and VIII into more active clotting factors (Va and VIIIa). These thrombin-activated factors accelerate the intermediate phases of coagulation, leading to a rapid generation of more thrombin, and subsequent formation of fibrin. One of the intermediate phases is the reaction of factor VIIIa with the activated form of factor IX (IXa). In the presence of phospholipid and calcium ions factors VIIIa and IXa convert factor X into an activated form⁴, which in turn reacts with factor Va, phospholipids and calcium ions to give prothrombin-converting activity^{5,6}. Factor IX is activated during the initial phases of blood coagulation, which involves activation of the Hageman factor (factor XII) as a result of contact with a foreign surface, and the activating action of the latter on factor XI⁷.

So far the activation of clotting factors by thrombin has been studied only with more or less purified preparations. It seemed to us that a more physiological way of studying the activation of clotting factors would be to investigate the dynamic process of activation and inactivation in slowly coagulating, recalcified citrated plasma. In this way we were able to determine the thrombin activation of factors VIII and V, the generation of activated factors IX, X and of thrombin, the disappearance of the activated factors and the sequence of the reactions involved.

Normal human plasma was obtained by centrifuging citrated blood (1 volume of trisodium citrate 3.8% + 9 volumes of blood), immediately after venepuncture, for 4 min at 12,000g at room temperature. The platelet-poor supernatant plasma was then centrifuged at 40,000g for 30 min at 0° C. To obtain plasma with a very slow and low thrombin generation, we had to work very quickly so as not to damage the platelets. Otherwise the activation of the clotting factors would have been too fast to be determined and the measurement of their activities would have been disturbed by the thrombin that was generated. The activities of clotting factors were measured by one-stage procedures⁸⁻¹⁰.

We introduced an important modification to the assays for factors VIII and IX, because of the great lability of activated

factor VIII. The diluted sample was omitted from the incubation mixture, but was added immediately after dilution, simultaneously with CaCl₂. The presence of fibrin monomers in solution was tested by the ethanol gelation test according to Godal¹¹. The thrombin generated was tested with a normal plasma sample (0.1 ml. of incubation mixture added to 0.1 ml. of citrated plasma), the clotting time of this being a measure for the thrombin concentration. The amount of thrombin generated was calculated on a calibration curve, for the determination of which we used NIH thrombin and the same normal plasma as substrate.

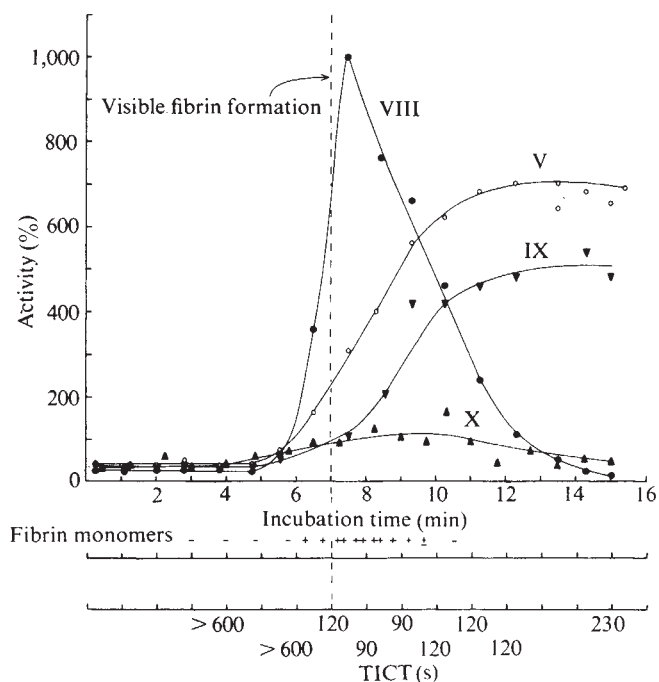


Fig. 1 Activation of clotting factors in a system consisting of equal amounts of practically platelet-free plasma, saline and CaCl₂, measured from the moment of recalcification. The formation of fibrin monomers, the appearance of a visible clot and the generation of thrombin, measured as the clotting time of normal plasma, are shown. □ TICT, Thrombin induced clotting time.

We investigated the activation of factors VIII, V, IX and X, the presence of fibrin monomers in solution, the appearance of the visible fibrin polymer (clotting of the mixture) and thrombin generation in a mixture of equal amounts of normal plasma, saline and CaCl₂ M/30, incubated at 37° C. The moment of recalcification was taken as zero time. At different times after recalcification, a sample was pipetted off, diluted 1 : 40 in Michaelis buffer (pH 7.25 μ = 0.15) and simultaneously assayed for factor VIII, IX and V activity by three people. Thrombin generation was assayed in an undiluted sample. Immediately afterwards another sample of the incubation mixture was recalcified and assayed for factor X activity after dilution 1 : 10 in the same buffer. This procedure was repeated for the detection of fibrin monomers. The clotting time and thrombin generation curve were the same for all three incubation mixtures (combined results are shown in Fig. 1). The activity of the clotting factors was normal until 5 min after recalcification, when there was a large and rapid activation of factor VIII. We noted an activation from 28% to 1,000%, corresponding to a change in clotting time of the assay from 100 s to 71 s for a dilution of 1 : 40. Factor V was activated simultaneously; at the maximum the clotting time was shortened from 43 s to 20 s, corresponding to an activation from 42% to 700%. For the third factor which was

activated, factor IX, there was a shortening of clotting time from 134 s to 92 s for a dilution of 1:40, corresponding to an activation from 40% to 500%. One minute before the coagulation of the mixture, fibrin monomers were detectable; visible fibrin formation lasted for 3 min, during which the test for fibrin monomers remained positive. Factor VIII activity disappeared from the clotted plasma very quickly, while high factor V and factor IX activities could be determined 2 h after clotting. In this clotting system the activity of factor X does not increase remarkably, the clotting time of a 1:10 dilution being shortened from 50 s to 40 s. The thrombin generation in this almost platelet-free plasma is very low. At the maximum of the thrombin generation curve only 0.3 U of thrombin/ml. are generated. This quantity of thrombin does not influence the determination of clotting factors, as blank tests proved.

The problem how the very first trace of thrombin, which initiates the autocatalytic activation of factors VIII and V, is generated, remains to be solved.

This work was supported by the Netherlands Foundation for Chemical Research with financial aid from the Netherlands Organization for the Advancement of Pure Research.

CATHERINE L. JANSSEN
WILHELMINA H. VAN LEEUWEN

Central Laboratory of the Netherlands Red Cross,
Blood Transfusion Service,
Amsterdam

Received February 23; revised April 19, 1971.

- ¹ Ware, A. G., Murphy, R. C., and Seegers, W. H., *Science*, **106**, 618 (1947).
- ² Lewis, M. L., and Ware, A. G., *Blood*, **9**, 520 (1954).
- ³ Rapaport, S. I., Schiffman, S., Patch, M. J., and Ames, S. B., *Blood*, **21**, 221 (1963).
- ⁴ Hemker, H. C., and Kahn, M. J. P., *Nature*, **215**, 1201 (1967).
- ⁵ Hemker, H. C., Esnouf, M. P., Hemker, P. W., Swart, A. C. W., and Macfarlane, R. G., *Nature*, **215**, 248 (1967).
- ⁶ Deggeller, K., and Vreeken, J., *Thromb. Diath. Haem.*, **22**, 45 (1969).
- ⁷ Ratnoff, O. D., Davie, E. W., and Mallett, D. L., *J. Clin. Invest.*, **40**, 803 (1961).
- ⁸ Stormorken, H., *Sc. J. Clin. Lab. Invest.*, **9**, 273 (1957).
- ⁹ Bachmann, F., Duckert, F., and Koller, F., *Thromb. Diath. Haem.*, **2**, 24 (1958).
- ¹⁰ Velkamp, J. J., Drion, E. F., and Loeliger, E. A., *Thromb. Diath. Haem.*, **19**, 279 (1968).
- ¹¹ Godal, H. C., and Abildgaard, U., *Scand. J. Haematol.*, **3**, 342 (1966).

Interruption of SV40 Oncogenesis with Human Foetal Antigen

IMMUNIZATION of adult hamsters with irradiated foetal hamster or mouse cells produces strong immunity to SV40 cell challenge and simultaneously induces cytostatic (or C) antibody formation¹. Furthermore, adenovirus 31 tumorigenesis can be interrupted by a single injection of hamster foetal cells if administered to neonatally infected hamsters 3 weeks after birth and before the appearance of the tumour². SV40 viral oncogenesis could be similarly interrupted in male but not female hamsters². This work provides support for the view that foetal and tumour cells share common antigens and that oncogenesis involves retrogenic activation of early foetal antigen genes in adult cells^{3,4}.

If the concept that ontogeny repeats phylogeny is applicable at the molecular level, early foetal proteins from different vertebrates should resemble each other more than adult proteins do. This would not be surprising, for the organizational problems of early foetal life are quite similar in different species, whereas the adaptation required for adult life may differ widely. Thus if viral oncogenesis involves activation of

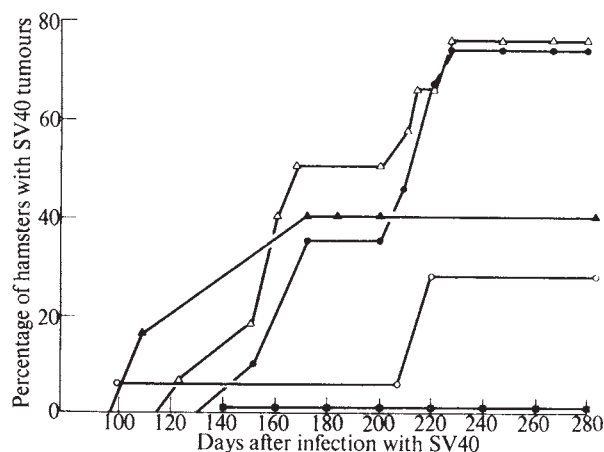


Fig. 1 Interruption of SV40 tumorigenesis by immunization with human embryonic kidney cells. Δ, No vaccine; ●, adult human kidney (5,000 r.); ▲, human embryonic kidney (5,000 r.); ○, SV40; ■, F5-1 SV40 tumour cells (5,000 r.).

specific foetal genes, these may be expected to be immunologically similar in cells of widely different origin. Conversely, foetal cells of different species might be expected to immunize across species barriers against tumours produced by the same virus. This is important if conclusions drawn from animal model studies are to be applied to man and if vaccination or immunotherapy involving specific antigens is to be attempted.

Hamster, mouse, and human cells transformed by SV40 virus all exhibit a similar antigen capable of stimulating specific transplantation immunity against SV40-induced tumours in hamsters^{5,6}, supporting the concept of foetal antigen similarity. We now report cross immunization of adult hamsters against SV40 virus tumorigenesis by human foetal cell homogenates.

SV40 hamster tumour cells (F5-1 line) were cultured as previously described⁷, and all experiments were done with the LVG hamster strain. C-Antibody levels were determined using implanted diffusion chambers containing SV40 tumour target cells^{1,2,7,8}.

SV40 virus (VA-45-54) was prepared in green monkey kidney cells (GMK) and had a titre of $10^{7.6}$ TCID₅₀/0.2 ml. in GMK. Hamsters were infected with 0.2 ml. of SV40 in the right subscapular space within 24 h of birth and palpated weekly for tumours after 4 weeks of age. Hamster foetal cell suspensions were prepared as previously described¹. Lyophilized cells were prepared from washed suspensions of freshly harvested (trypsin) F5-1 tumour cells in HBSS. The cells were reconstituted in sterile distilled water to the original volume of liquid which had originally contained 5×10^6 viable cells/ml. Primary human embryonic kidney cells were purchased from Flow Laboratories, Rockville, Maryland. Foetal immunogens and control materials were exposed to 5,000 r. of X-irradiation before injection¹. Five million trypan blue excluding cells were used for each immunization with all preparations except the lyophilized F5-1 cell vaccine.

Cytostatic antibody against SV40 tumour cells appears within 4 weeks after infection of neonate hamsters with SV40, persists in the peritoneal fluid and blood until several weeks before palpable tumour formation, then disappears⁹. SV40 tumours rarely appear in less than 90–100 days after infection. Neonatally infected hamsters were immunized with three injections of either SV40, irradiated SV40 hamster tumour cells, human embryo kidney cells, adult kidney cells, lyophilized SV40 tumour cells, 10-day gestation hamster foetal cells, or nothing. The results in Table 1 show that at 56–60 days after infection, non-immunized SV40-infected animals possessed a significant level of C-antibody, as previously reported⁹. Immunization of infected hamsters with SV40 virus, irradiated F5-1 tumour cells, primary human embryo kidney cells, or hamster foetal cells always increased the C-antibody level over that observed in control animals receiving no immuniza-

tion. Adult primary human kidney cells, lyophilized SV40 tumour cells, or non-irradiated hamster foetal cells always diminished the C-antibody level below that observed in non-immunized SV40 virus infected control animals (Table 1). Lyophilized SV40 tumour cells, non-irradiated hamster foetal cells, or adult human cells failed to induce either immunity to SV40 tumour cell challenge or to interrupt SV40 oncogenesis. Fifty per cent protection against SV40 tumour induction was observed among hamsters immunized with irradiated human embryo kidney cells, and 62% protection was afforded by immunization of adult hamsters with SV40 (Fig. 1). Irradiated SV40 tumour cells gave complete protection. We have previously demonstrated that irradiated hamster foetal cells protect male hamsters against SV40 tumorigenesis².

Table 1 Potentiation of Diminution of Cytostatic Antibody Level to SV40 Hamster Tumour Cells using Human Foetal Cells as Immunogen in SV40-Infected Male Hamsters

Neonatal inoculation *	Immunogen †	Average No. of viable cells/ chamber ‡	Inhibition § (%)
Passage control fluid	None	626,000	Control
SV40	None	441,000	30
SV40	SV40	387,000	39
SV40	F5-1 (5,000 r.)	344,300	45
SV40	Adult human kidney cells	476,400	23
SV40	Primary human embryonic kidney cells	309,700	51
SV40	Lyophilized F5-1 (5,000 r.)	594,700	5
SV40	10 day hamster foetus (5,000 r.)	344,400	45
SV40	10 day hamster foetus (0 r.)	565,800	10

* Newborn hamsters were infected with SV40 within 24 h after birth or given noninfected cell culture control fluid.

† SV40 or irradiated cells (5,000 r.) administered intraperitoneally at 3 weeks of age, weekly for 3 weeks.

‡ Fifteen chambers originally inoculated with 18,000 viable SV40 tumour cells, implanted in immunized 56 day old hamsters for 5 days, and enumerated by trypan blue exclusion test.

§ Percentage inhibition of SV40 target cell growth in chambers observed between immunized control animals. All values given were significantly different from control at 1% confidence level as determined by Wilcoxon test.

The results show that injection of control tissues, lyophilized SV40 tumour cells, or non-irradiated hamster embryo tissues diminishes detectable levels of circulating antibody to the SV40 tumour target cells in the diffusion chambers. In contrast, human foetal cells and irradiated hamster foetal cells increase the level of circulating C-antibody, supporting the view that SV40-induced TSTA is the result of the retrogenic expression of a foetal or embryonic antigen gene.

Research was conducted under the joint NIH-AEC Molecular Anatomy (MAN) Program supported by the National Cancer Institute, the National Institute of General Medical Sciences, the National Institute of Allergy and Infectious Diseases, and the US Atomic Energy Commission.

K. R. AMBROSE
N. G. ANDERSON
J. H. COGGIN

*Molecular Anatomy (MAN) Program,
Oak Ridge National Laboratory,
Oak Ridge,
Tennessee 37830, and
Department of Microbiology,
University of Tennessee,
Knoxville,
Tennessee*

Received April 16, 1971

- Coggin, J. H., Ambrose, K. R., and Anderson, N. G., *J. Immunol.*, **105**, 524 (1970).
- Coggin, J. H., Ambrose, K. R., Bellomy, B. B., and Anderson, N. G., *J. Immunol.* (in the press).
- Anderson, N. G., and Coggin, J. H., *Science* (in the press).
- Potter, V. R., *Proc. Eighth Canadian Cancer Res. Cong.* (in the press).
- Girardi, A. J., *Proc. US Nat. Acad. Sci.*, **54**, 445 (1965).
- Kit, S., Kurimura, T., and Dubbs, D. R., *Intern. J. Cancer*, **4**, 384 (1969).
- Ambrose, K. R., Candler, E. L., and Coggin, J. H., *Proc. Soc. Exp. Biol. Med.*, **132**, 1013 (1969).
- Coggin, J. H., and Ambrose, K. R., *Proc. Soc. Exp. Biol. Med.*, **130**, 246 (1969).
- Ambrose, K. R., and Coggin, J. H., *Bact. Proc.*, 187 (1970).

Correlation between Humoral Antibody and Regression of Tumours induced by Feline Sarcoma Virus

FELINE sarcoma virus (FSV) induces malignant tumours in cats¹⁻⁴, dogs^{1,2,4}, rabbits^{1,4} and monkeys^{4,5}, and transforms cultured cells from several species including man^{6,7}. Spontaneous regression of tumours induced by FSV has been reported³. We describe here a correlation between the presence of humoral antibodies to virus-associated cell membrane antigens and the failure of cats injected with FSV to develop progressive malignant tumours.

An indirect membrane immunofluorescence technique similar to that described for the detection of membrane antigens in murine leukaemia cells was used⁸. A lymphoblastoid cell culture derived from a feline leukaemia virus (FeLV)-induced tumour provided target cells. The derivation and maintenance of this culture, which produces FeLV in a steady state manner, has been described before⁹. Fluorescein-conjugated antiserum to cat IgG prepared in a goat (obtained from Dr Robert Holdenreid, National Cancer Institute, Bethesda), or rabbit (obtained from Dr Edwin Lennette, California State Department of Public Health, Berkeley) was used at a 1 : 20 dilution.

Bright and dense sectorial or ring fluorescence was observed in 90-100% of the cells at serum dilutions up to 1 : 128 with serum from certain regressor cats (Fig. 1). Two-fold dilutions of each serum sample were prepared, and fifty cells were examined at each dilution until fluorescence was no longer observed. The serum dilution at which the number of fluorescing cells had decreased to 50-75% usually was the same dilution at which punctate rather than ring or sectorial fluorescence was observed. We decided to designate end-point titres as the reciprocal of the highest dilution where 50% or more of the cells had distinct spots of fluorescence. The negative serum samples caused no such fluorescence even when undiluted. All samples were coded and read blind.

Serum samples were taken from cats before the injection of FSV and between 3 and 7 weeks later. Cats with progressive tumours usually died within 3-5 weeks after FSV inoculation and so later samples could only be obtained from regressor cats or those that failed to develop palpable tumours. Cats which were 0-1, 4, 13, 26, and 52 weeks old received 1.0 g equivalents of FSV prepared as previously described³. The inverse relationship between increasing age at the time of FSV injection and the incidence of progressive malignant tumours has been previously observed (S. P. S., manuscript in preparation), and the gross morphology and histopathology of progressive and regressive tumours has been described³.

A distinct negative correlation was seen between the antibody titre 3-7 weeks after FSV inoculation and the development of progressive tumours (Table 1). Of three cats which were injected with FSV at 1 yr of age, two failed to develop palpable tumours and one developed a tumour which subsequently

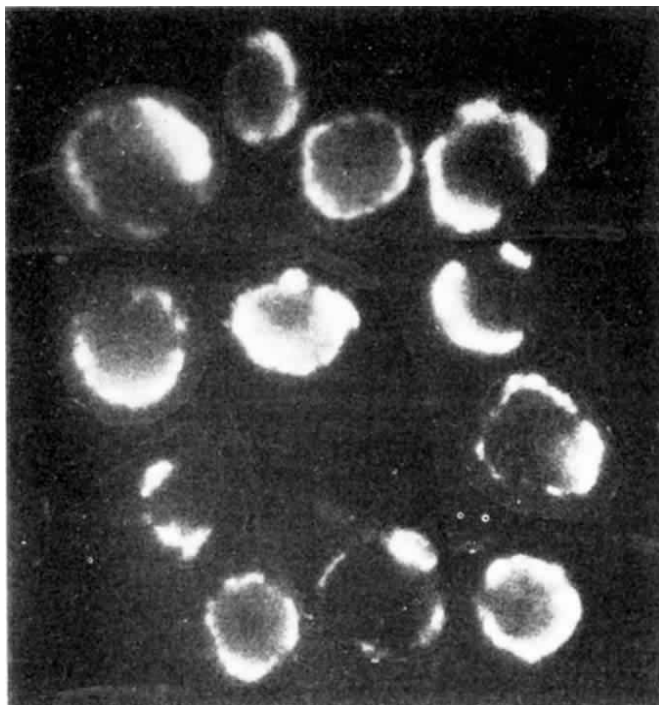


Fig. 1 Feline leukaemia virus-producing lymphoblastoid cells showing ring membrane fluorescence. The cells were exposed to serum (1:16 dilution) from a cat with a regressing FSV-induced sarcoma and sarcoma and subsequently to fluorescein labelled goat anti-cat IgG (1:20 dilution).

regressed. All three developed antibody titres which ranged from 16 to 256. The only kitten given FSV at 0-1 week of age which manifested tumour regression was also the only one in this age group to develop a significant humoral antibody titre. When all age groups are considered, nine cats developed tumours that were classified as progressive. Of these nine, none developed antibody titres above 2, and five had no demonstrable antibody. Twelve cats either developed no tumour or developed tumours which subsequently regressed. All twelve developed

Table 1 Titres of Antibody Detected by Indirect Immunofluorescence in Serum Samples from Cats of Different Ages Inoculated with FSV

Cat No.	Age in weeks*	Antibody titres-weeks†					Type of tumour response‡
		0	3	4	5	7	
1	52	0	128			128	regressor
2	52	0	4			128	no tumour
3	52	0	0			16	no tumour
4	26	0	16				regressor
5	26	16	64				regressor
6	26	0	2				regressor
7	26	0	32				regressor
8	13	0		0			regressor
9	13	8	64	256	256		regressor
10	13	0	2				regressor
11	13	8	8	32	16		regressor
12	13	0	0				regressor
13	13	0		1			regressor
14	4	0	4				regressor
15	4	0	4				regressor
16	4	0	4				regressor
17	0-1	0	0				regressor
18	0-1	0	1				regressor
19	0-1	0	4				regressor
20	0-1	0	0				regressor
21	0-1	0	0				regressor

* Age at time of FSV inoculation.

† Weeks following FSV inoculation.

‡ See text for definition of regressor and progressor.

antibody titres of 4 or higher and eight had titres of at least 16 (Tables 1 and 2). Regressor tumours are defined as those which show a visible reduction in size before 7 weeks after FSV injection. Progressor tumours are those which show no visible or palpable regression, and which debilitate the host to the point of death. Kittens with progressive tumours were terminally exsanguinated to obtain serum. The reason for the presence of antibody titres in three of the twenty-one cats before FSV inoculation is not known, but the horizontal transmission of oncornaviruses in laboratory cat colonies has been reported¹⁰, and since these three cats were either 13 or 26 weeks old at the time of testing, it is possible that they had been exposed to either FSV or FeLV.

Table 2 Summary of Antibody Response of Cats of All Ages According to Tumour Response and Antibody Titres

Type of tumour response	No. of cats with titres of 4 or over	No. of cats with titres under 4
No tumour	2	0
Regressors	10	0
Progressors	0	9
Total	12	9

The findings reported here clearly demonstrate an association between the development of antibody titres and either tumour regression or the lack of palpable tumour development. They also demonstrate a reaction between antibody associated with injections of FSV and a cell membrane antigen of cells that produce FeLV.

We thank Drs Edwin Lennette and Robert Holdenreid for fluorescein-conjugated antiserum, Harriet Blomquist and Sharon Beall for technical assistance, and Dr Leo Bustad for encouragement and criticism. This work was supported by fellowship awards from the Leukemia Society of America (M. E.) and the American Cancer Society (S. P. S.) as well as general support from the Special Virus-Cancer programme of the National Cancer Institute, the US National Institutes of Health and Public Health Service, the Swedish Cancer Society, the Jane Coffin Child's Memorial Fund for Medical Research, and the Harald and Greta Jeansson Foundation.

MYRON ESSEX
GEORGE KLEIN

*Department of Tumour Biology,
Karolinska Institute School of Medicine,
Stockholm*

STANLEY P. SNYDER
J. BOYD HARROLD

*Comparative Oncology Laboratory,
University of California,
Davis*

Received March 31; revised May 28, 1971.

- Snyder, S. P., and Theilen, G. H., *Nature*, **221**, 1074 (1969).
- Gardner, M. B., Rongey, R. W., Arnstein, P., Estes, J., Sarma, P., Huebner, R. J., and Rickard, C. G., *Nature*, **226**, 807 (1970).
- Snyder, S. P., Theilen, G. H., and Richards, W. P. C., *Cancer Res.*, **30**, 1658 (1970).
- Theilen, G. H., Snyder, S. P., Wolfe, L. G., and Landon, J. C., *Comparative Leukemia Research* (edit. by Dutcher, R. M.), (Karger, New York, in the press).
- Deinhardt, F., Wolfe, L. G., Theilen, G. H., and Snyder, S. P., *Science*, **167**, 881 (1970).
- Sarma, P. S., Huebner, R. J., Basker, J. F., Vernon, L., and Gilden, R. V., *Science*, **168**, 1098 (1970).
- Chang, R. S., Golden, H. D., and Harrold, B., *J. Virol.*, **6**, 599 (1970).
- Klein, E., and Klein, G., *J. Nat. Cancer Inst.*, **32**, 547 (1964).
- Theilen, G. H., Kawakami, T. G., Rush, J. D., and Munn, R. J., *Nature*, **222**, 589 (1969).
- Rickard, C. G., *J. Small Animal Practice*, **10**, 615 (1969).

Inhibiting Effect of some Antimalarial Substances on Glucose-6-phosphate Dehydrogenase

COMPOUNDS with antimalarial activity are used as therapeutic agents in a number of disorders other than malarial infections. Our dermatological interest in these substances is two-fold. First, they are specific therapeutics for a number of autoimmune disorders with cutaneous manifestations¹ and, second, the use of these substances in malarial therapy or prophylaxis often causes adverse cutaneous side effects¹.

It has been known for some time that individuals with an erythrocytic glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) deficiency (favism) have an increased resistance to malaria². These individuals also have a high incidence of haemolytic anaemia when given antimalarial drugs prophylactically². These observations suggest that antimalarial drugs inhibit erythrocytic glucose-6-phosphate dehydrogenase (G6PDH) in malaria. Analogously, they may inhibit the G6PDH activity of the reticulo-endothelial system in their capacity as immunosuppressants, and that of the skin in their production of cutaneous manifestations. An essential first test of such an hypothesis is to see if these compounds are capable of such inhibition *in vitro*. We have therefore tested a range of antimalarial substances on purified yeast G6PDH from Sigma and on whole tissue extracts of erythrocytes and skin.

The usual commercial forms of the antimalarial substances were dissolved and filtered before use. Enzyme was assayed at room temperature by following the formation of NADPH fluorimetrically (excitation 340 nm, fluorescence 460 nm), or spectrophotometrically when difficulties from quenching were experienced. The general details of the assay have been described by Halprin and Ohkawara³. The reaction mixtures used are given in Table 1.

Table 1 Reaction Mixture for Testing Inhibitors of Glucose-6-phosphate dehydrogenase

	NADP 0.002 M in buffer (ml.)	G6PDH 10 ⁻³ mg/ml. in buffer (ml.)	Buffer 0.05M Tris (pH 7.5) + 5 × 10 ⁻⁴ M EDTA + 0.03 M MgCl ₂ (ml.)	Inhibitor 10 ⁻³ M in buffer (ml.)	G6P (diNa) 0.014 M in buffer (ml.)
1	0.10	0.10	1.60	0.10	0.10
2	0.10	0.10	1.69	0.10	0.10
3	0.10	0.10	1.69	0.10	0.01

Before calculating the results it was observed that the inhibition could be reversed completely in most cases by excess NADP, that is, the inhibition was competitive for NADP. Therefore the following equation was used to calculate the K_i values

$$K_i = \frac{V_i}{V_0 - V_i} \times \frac{Km}{Km + [I]}$$

where V_i is the initial reaction velocity in the presence of the inhibitor, V_0 the initial velocity of the reaction in the absence of inhibitor, $[I]$ the final concentration of inhibitor, Km the Michaelis-Menton constant, K_i the inhibition constant and $[S]$ the concentration of substrate (NADP). This equation was derived by dividing the normal Michaelis-Menton equation by the equation for a competitively inhibited reaction and rearranging to give K_i .

In normal Lineweaver-Burk plots, the Km for glucose-6-phosphate and NADP were found to be 6.9×10^{-5} M and 1.43×10^{-5} M respectively. The enzyme was specific for NADP, which could not be replaced by NAD, and the reaction was inhibited by NADPH. The results of the inhibition studies are shown in Table 2.

Table 2 Inhibition of G6PDH by Antimalarials

Inhibitor	% inhibition at low [NADP]	% inhibition at low [G-6-P]	% inhibition at high [NADP] + [G-6-P]	Approximate K_i	Method used
Paludrine	30	—	—	1.1×10^{-4}	Fluorimetric
Daraprim	36.4	—	—	8×10^{-5}	Fluorimetric
Mepacrine	17.2	—	—	1.8×10^{-3}	Spectrophotometric
Primaquine	28.1	—	—	1.2×10^{-3}	Spectrophotometric
Chloroquine	47.5	—	—	5.14×10^{-4}	Spectrophotometric

The antimalarial compounds also inhibited the G6PDH reaction in skin and erythrocyte extracts to a similar extent. Gold salts were found to inhibit skin G6PDH noncompetitively and streptomycin to inhibit competitively for glucose-6-phosphate. Both of these compounds have a similar therapeutic application and range of side effects as the antimalarials proper¹.

It is interesting to consider the consequences of such an inhibition to the metabolism of the cell. G6PDH is the rate limiting point of entry into the pentose phosphate shunt which is the sole producer of extramitochondrial NADPH and also produces riboses for nucleic acid synthesis. Obviously inhibition of this enzyme and pathway will have far reaching effects, particularly in subjects such as favists who have a reduced G6PDH activity in the first place. It would also be of great interest to know if subjects who experience lichenoid skin eruptions as a side effect of antimalarial therapy have a skin-specific deficiency in this enzyme. We are currently carrying out such investigations.

We thank Professor J. W. H. Mali, Dr P. D. Mier and Dr R. C. A. Sengers for helpful discussions.

D. W. K. COTTON
A. H. M. SUTORIUS

Department of Dermatology,
Catholic University,
Javastraat 104, Nijmegen

Received April 6, 1971.

¹ Rook, A., Wilkinson, D. S., and Ebling, F. J. G., in *Textbook of Dermatology*, 2, 1209 (Blackwell, Oxford/Edinburgh, 1968).

² Halprin, K. M., and Ohkawara, A., *J. Invest. Dermatol.*, **46**, 43 (1966).

³ Harris, H., *The Principles of Human Biochemical Genetics* (Frontiers of Biology, series 19) (North-Holland, Amsterdam, 1970).

Effect of Infection with Trypanosomes on the Development of Experimental Allergic Neuritis in Rabbits

DATA obtained from hospitals in western Nigeria¹ have shown that rheumatoid arthritis is uncommon in that region and, when the disease occurs, it is clinically milder than in Europeans, involving few of the expected immunological changes^{2,3}. Other conditions in which auto-immune processes are thought to be involved were also found to be rare, and Greenwood suggested that repeated exposure to parasitic infections might be responsible, at least in part, for the infrequent occurrence of these auto-immune diseases. Infection with the rodent malaria parasite *Plasmodium berghei yoelii* delayed the onset

of haemolytic disease in 4 week old NZB mice and prevented the development of renal disease in (NZB×NZW) F₁ mice at the same age⁴. The severity of adjuvant arthritis in rats was also reduced by simultaneous inoculation of *P. berghei yoelii*, which produces a very mild infection, but not by infection with a more virulent strain of *P. berghei berghei*⁵. We are investigating the effects of trypanosome parasites on the development in rabbits of experimental allergic neuritis (EAN), a demyelinating paralytic disease⁶, the genesis of which is primarily a cell-mediated type of immune response (manuscript in preparation), and the preliminary results are reported here.

Adult New Zealand white rabbits of both sexes were infected by intraperitoneal inoculation of blood from mice infected with trypanosome parasites; five received *Trypanosoma brucei* (Serial No. LUMP 143) which is maintained as a stabilate⁷, and seven received a *T. brucei* parasite (strain S 427 supplied by Dr L. G. Goodwin and now maintained as stabilate LUMP 231). Parasitaemia was estimated by examination of fresh blood preparations by phase contrast microscopy (×40 objective) every 2–3 days. The rabbits infected with *T. b. brucei* LUMP 143 were cured 24 days after inoculation of parasites by treatment with 'Berenil' (10 mg/kg given intramuscularly). Five of the seven animals infected with *T. brucei* S 427 were cured in the same way 59 days after inoculation; the other two developed severe EAN signs and had been killed and autopsied by this time.

Extraneous fat and connective tissue were removed from human sciatic nerve obtained at *post mortem*. The nerve was cut on a freezing microtome and the "brei" produced was homogenized with saline. An equal volume of Freund's complete adjuvant was added and a stable water-in-oil emulsion prepared by further homogenization. 0.2% of phenol was added to the antigen which was stored at -20° C. 0.2 ml. of antigen were injected intradermally into the left hind foot pad of each rabbit. Further details of antigen preparation and injection procedure are given elsewhere⁸.

The antigen was injected into nine uninfected rabbits and into the twelve trypanosome-infected animals 19 or 21 days

after inoculation of parasites. After the injection of EAN antigen, animals were examined by two observers at 2 day intervals for signs of functional disability. Autopsy was carried out at 55 days after antigen challenge or earlier when necessitated by the onset of severe paralysis. Rabbits were killed by intravenous injection of 'Nembutal'. For preliminary histological survey, dorsal lumbar roots were removed and fixed with osmium tetroxide. Teased preparations were made from these nerves as previously described⁸.

Live parasites were seen in all rabbits during the period between inoculation of trypanosomes and injection of EAN antigen. Since the levels of infection at this stage were so low (one to ten trypanosomes per fifty microscope fields), no importance was attached to the apparent length of the latent period; the shortest was 3 days and the longest was 14 days, the average being about 7 days. *T. b. brucei* LUMP 143 infections had reached a level of one to fifteen parasites per microscope field by day 24 and were therefore treated, but *T. brucei* S 427 was less virulent.

EAN signs were assessed on a three point scale. Early and mild cases (denoted by +) showed features such as weakness of hind limb(s), poor "displacement reflex", some ataxia, some sliding of fore limbs and poor "withdrawal reflex".

In later stages (++) these features became more pronounced and more frequent. Severe cases (+++) showed additional signs such as extended flaccid limbs, great difficulty in walking, flailing of limbs, total limb paralysis, incontinence, loss of appetite and weight and laboured breathing (for more details of EAN signs see Allt, Evans and Evans⁸).

In the "EAN+trypanosomiasis" group (twelve rabbits) seven animals were alive 55 days after EAN antigen challenge. Five of these had shown no overt EAN signs. Of the "EAN alone" group (nine rabbits) only one remained alive at 55 days after injection of EAN antigen, and it had previously shown unambiguous EAN signs. The remaining rabbits of this group developed marked (++) or severe (+++) EAN signs and had to be killed (Table 1).

Spinal roots are especially vulnerable to demyelination in EAN and lumbar dorsal roots were chosen for preliminary

Table 1 EAN Signs (Severity, Duration, Onset) and Histopathology

	Animal	Severity of EAN signs§	Day of onset of overt EAN signs (after EAN antigen challenge)	Duration of EAN signs	Survival* (at 55 days after EAN antigen challenge)†	Histology‡ (lumbar dorsal roots)	
						Demyelination	Remyelination
EAN in trypanosome-infected rabbits	1	None	—	—	Yes	—	—
	2	None	—	—	Yes	t	—
	3	None	—	—	Yes	—	t
	4	None	—	—	Yes	—	t
	5	None	—	—	Yes	—	—
	6	+	28	12	Yes	+	++
	7	++	19	21	Yes	+++	++
	8	+++	14	2	No	++	—
	9	++	15	8	No	+	—
	10	+++	13	2	No	++	—
	11	+++	14	2	No	+	—
	12	++	35	3	No	+++	—
EAN in uninfected rabbits	13	+	23	17	Yes	+	t
	14	+++	14	2	No	++	—
	15	+++	18	5	No	+++	—
	16	+++	10	6	No	+++	—
	17	+++	18	3	No	++	—
	18	+++	15	7	No	t	—
	19	+++	18	12	No	++	—
	20	++	14	2	No	t	—
	21	+++	13	3	No	+++	—

* Rabbits showing (a) severe paralysis (+++), (b) marked paralysis (++), together with respiratory difficulty, were killed.

† Difference in survival between the two groups is significant, that is, greater than two standard errors (s.e. 0.22) or $P < 0.05$.

‡ Four point scale for extent of demyelination/remyelination derived from teased preparations: +++, "extensive"; ++, "moderate"; +, "occasional"; t, "trace".

§ See text for grading of EAN signs.

examination. Teased preparations of osmicated nerves from paralysed rabbits revealed focal, segmental myelin loss characteristic of EAN. Various stages of myelin breakdown could be observed, ranging from the replacement of a part of an internode by several large myelin ovoids, through many droplets of myelin, to totally denuded axon segments⁸. Remyelination, in the form of short, intercalated internodes, was evident in animals which had recovered from EAN. Unlike the adjacent undamaged myelin, such internodes had thin myelin sheaths.

Table 1 correlates the severity of EAN signs and the extent of histological changes. Generally, the greater the paralysis and ataxia the more extensive the demyelination. Occasionally marked paralysis was accompanied by only sparse demyelination. The explanation for this may be either: (a) inadequate histological sampling, or (b) that demyelination is not the only important histopathological effect. Comparisons of the time of onset and the duration of EAN in control rabbits and in those trypanosome-infected animals which were affected showed no significant differences between the two groups.

In this series of experiments, all the control animals developed unequivocal signs of the disease whereas a significantly smaller number of the trypanosome-infected animals did so. It seems probable therefore that the presence of the parasite caused a marked suppression of EAN. We cannot say at present what the mechanism of this suppression is but it might have an immunological basis since EAN induction is primarily a cell-mediated immune response (manuscript in preparation). The effects of trypanosome infections on such responses have not to our knowledge been investigated, but Goodwin *et al.*⁹ have examined the antibody response to sheep erythrocytes in mice and rabbits infected with *T. brucei*. There was a pronounced depression of the anti-sheep red blood cell response in the infected mice and immunodepression was also demonstrable in some of the trypanosome-infected rabbits. The response in other rabbits was very difficult to interpret, however, because of the appearance during the infection of heterophile antibody which reacted with antigen of the sheep erythrocytes (Goodwin and Voller, personal communication). They suggested that the presence of the trypanosomes seemed to make it difficult for the rabbit to produce antibody to "large" antigens which may require to be processed by macrophages before antibody forming cells can be stimulated.

It is interesting that the trypanosome infection suppressed EAN in some of our rabbits but was ineffective in others in terms of the time of onset and duration of EAN symptoms and the histological reactions. These variations in response to EAN were not related to the strain of parasite used and could not be correlated with any observed feature of the trypanosome infections. Thus suppression was not obviously related to the overall level of parasitaemia or to "peaks" of infection such that the results might be explained in terms of antigenic competition due to the number of parasites present. It is important to remember that the trypanosome is a tissue parasite¹⁰ and that there are likely to be many more trypanosomes in the extravascular spaces than in the peripheral circulation. We are investigating the effect of varying the time of parasite infection in relation to antigen injection on the subsequent development of EAN.

G. ALLT
E. M. E. EVANS
D. H. L. EVANS

Department of Anatomy,
University College London,
Gower Street,
London WC1

G. A. T. TARGETT

Department of Medical Protozoology,
London School of Hygiene and Tropical Medicine,
Keppel Street,
London WC1

Received June 4; revised July 2, 1971.

- ¹ Greenwood, B. M., *Lancet*, ii, 380 (1968).
- ² Greenwood, B. M., *Ann. Rheum. Dis.*, **28**, 488 (1969).
- ³ Greenwood, B. M., and Herrick, E. M., *Brit. Med. J.*, **1**, 71 (1970).
- ⁴ Greenwood, B. M., Herrick, E. M., and Voller, A., *Nature*, **226**, 266 (1970).
- ⁵ Greenwood, B. M., Voller, A., and Herrick, E. M., *Ann. Rheum. Dis.*, **29**, 321 (1970).
- ⁶ Waksman, B. H., and Adams, R. D., *J. Exp. Med.*, **102**, 213 (1955).
- ⁷ Lumsden, W. H. R., and Hardy, G. J. C., *Nature*, **205**, 1032 (1965).
- ⁸ Allt, G., Evans, E. M. E., and Evans, D. H. L., *Brain Res.*, **29**, 271 (1971).
- ⁹ Goodwin, L. G., *Trans. Roy. Soc. Trop. Med. Hyg.*, **64**, 797 (1970).
- ¹⁰ Ormerod, W. E. in *The African Trypanosomiasis* (edit. by Mulligan, H. W., and Potts, W. H.) (Allen and Unwin, 1970).

¹³C NMR Spectra of Lecithin Vesicles and Erythrocyte Membranes

UNLIKE proton nuclear magnetic resonance (NMR) spectroscopy, ¹³C NMR has the potential advantages in studies of macromolecular systems of a wider range of chemical shifts (~200 p.p.m.) and of reduced resonance broadening in structures with restricted molecular motion. Thus we have been able to observe ¹³C NMR spectra of unsonicated lecithin and erythrocytes, which do not yield high resolution proton spectra. We also report *T*₁ relaxation times for ¹³C nuclei in the polar headgroup of the lecithin molecule and at both ends of the alkyl chains, in sonicated vesicles in D₂O buffer and in deuteriochloroform. The results are consistent with increasing chain motion towards the terminal methyl group of the alkyl chains for both systems, in agreement with relaxation data for stearic acid derivatives specifically labelled with ¹⁹F nuclei in lecithin vesicles¹, and with the results of similar spin label experiments of Hubbell and McConnell².

Dipalmitoyl lecithin was obtained from Koch Light, and human erythrocyte membranes were prepared as described previously³. Lecithin (230 mM) was sonicated in D₂O buffer containing 45 mM NaCl, 30 mM Na acetate and 5 mM Na phosphates (pH 7.4) in glass vials under nitrogen at 50° C until translucent, and transferred into 12 mm NMR tubes under nitrogen. The ¹³C NMR spectra were obtained by the Fourier transform technique⁴ on a Varian XL 100-15 spectrometer at 25.1 MHz locked onto deuterium in the solvent (D₂O or CDCl₃), and accumulated in a Varian 620i computer. Chemical shifts were corrected to a dioxan internal reference. A pulse method similar to that of Vold *et al.*⁵ (π - t - $\pi/2$ pulse sequence) and modified by Freeman and Hill (private communication) was used to measure *T*₁ relaxation times, where *t* is the delay in seconds between the π and $\pi/2$ pulses. The *T*₁ measurements were made on lecithin in D₂O buffer thoroughly deoxygenated with nitrogen, and CDCl₃ degassed by repeated freeze-pump-thaw cycles. Proton NMR spectra were obtained on a Varian HA100-15 spectrometer.

The ¹³C spectra of dipalmitoyl lecithin dissolved in CDCl₃ and a fully sonicated sample in D₂O buffer are shown in Fig. 1 *a, b*. The alkyl chain carbons were assigned by analogy with the published spectra of fatty acids⁶, and the other resonances by comparison with the spectra of the model compounds choline, choline glycerol phosphate, and phosphatidic acid. All the resonances in both systems are relatively sharp and even the signals from the alkyl chain carbons remain well resolved in the vesicle bilayers. This enabled *T*₁ measurements to be made on most of the observable nuclei in the molecule and the results are shown in Table 1; values shorter than 0.1 s were inaccessible in the present experiments. In both systems *T*₁ increases along the alkyl chains towards the terminal methyl

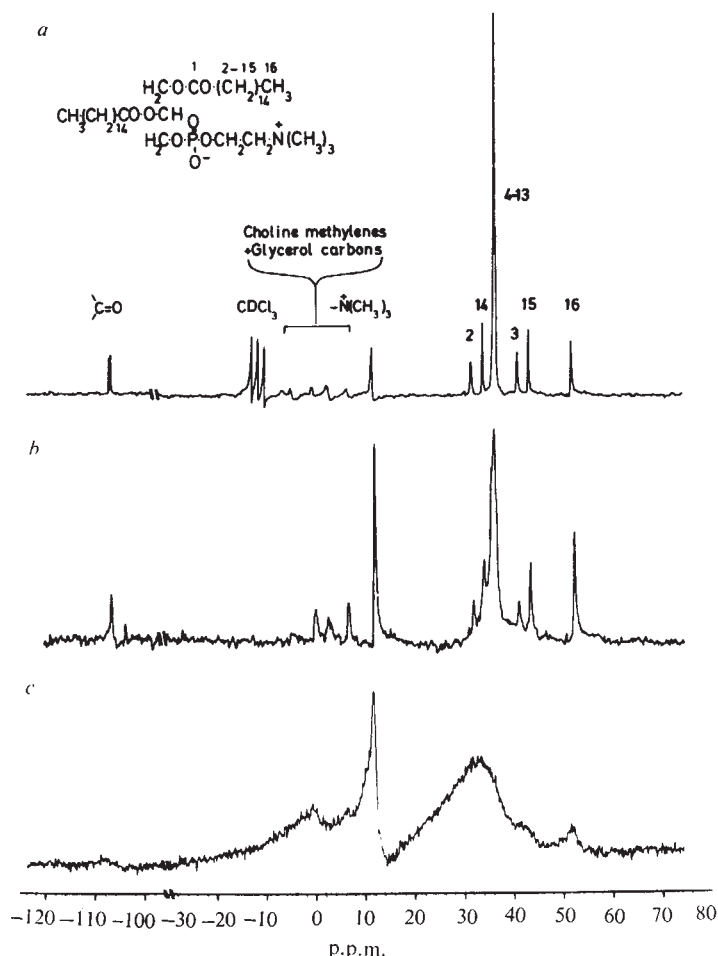


Fig. 1 The ^{13}C NMR spectra of dipalmitoyl lecithin. *a*, 290 mM lecithin in CDCl_3 at 28°C . Shifts are corrected to an internal dioxan reference of zero p.p.m. *b*, Fully sonicated and *c*, unsonicated lecithin (230 mM) in D_2O buffer at 52°C . (The unsonicated sample also contained 26 mM palmitic acid to prevent coagulation of the suspension; the palmitic acid did not affect the spectrum of the same sample in the sonicated state.)

group although there are significant differences in the gradation of the changes. A striking difference between the two systems is the very short T_1 value for the $\text{N}^+(\text{CH}_3)_3$ carbon nuclei relative to the $(\text{CH}_2)_n$ resonance envelope in CDCl_3 . The spectra after delays of 0.1 and 1.0 s between the π and $\pi/2$ pulses show this difference clearly in Fig. 2, in which the $\text{N}^+(\text{CH}_3)_3$ resonance has almost disappeared in CDCl_3 after 0.1 s but is still relatively large compared with the $(\text{CH}_2)_n$ resonance in the D_2O buffer. We suggest that the short T_1 for the $\text{N}^+(\text{CH}_3)_3$ nuclei in CDCl_3 may be due to the formation of a micellar structure with the polar headgroups restricted in motion by being at the centre of the micelle with the alkyl

chains exposed to the CDCl_3 solvent. In contrast, in the bilayer structure formed in water where the headgroups are exposed to the D_2O solvent, we expect relatively free motion of the $\text{N}^+(\text{CH}_3)_3$ group and a longer T_1 value. In both systems we attribute the changes in T_1 along the alkyl chain to increasing chain motion towards the terminal methyl group. By appropriate substitution in the alkyl chain of stearic acid, for example, with a single fluorine atom, or by the introduction of a double bond, at least twelve of the eighteen carbon nuclei can be resolved. Some of these extra resonances are resolved in egg lecithin containing unsaturated chains. Relaxation measurements on synthetic lecithins incorporating substituted and unsaturated fatty acids will be reported elsewhere.

Fig. 1c shows the ^{13}C spectrum of unsonicated lecithin in which both the $\text{N}^+(\text{CH}_3)_3$ and terminal methyl resonances remain relatively narrow in contrast to the main methylene envelope which is broadened approximately eight-fold. In

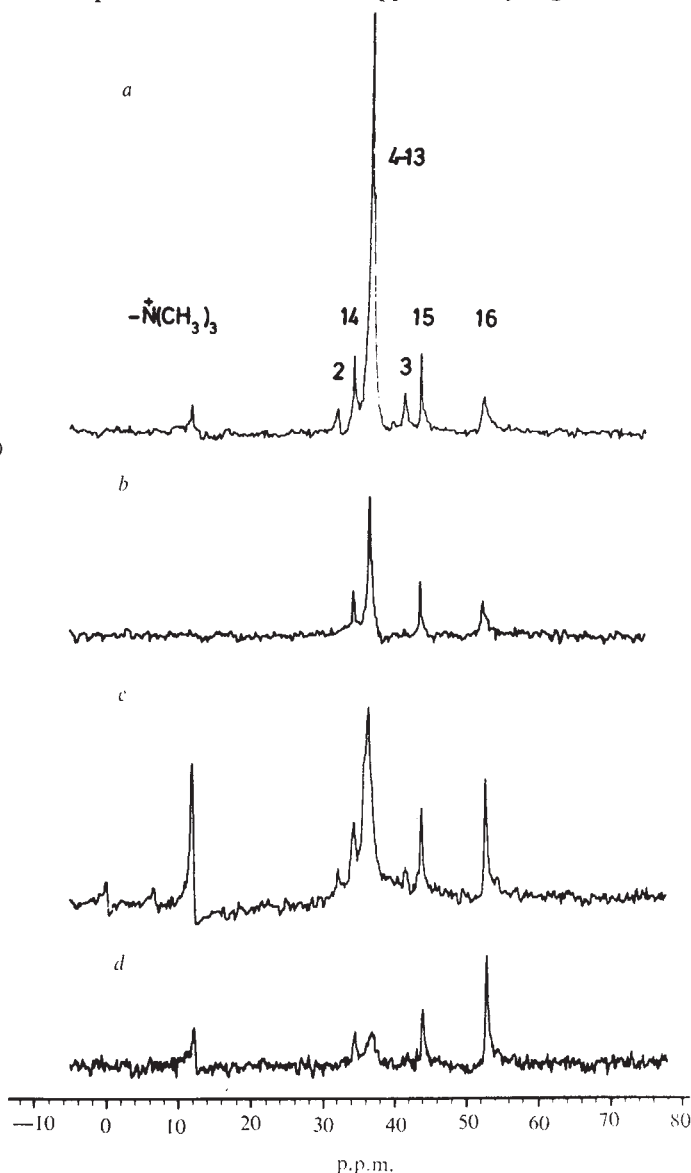


Fig. 2 The ^{13}C NMR spectra of dipalmitoyl lecithin with a pulse sequence $(\pi - t - \pi/2 - t_1 - \pi/2)$ where t is the delay between π and $\pi/2$ pulses and t_1 is a long delay (~ 20 s): the observed signal is the difference between the signals resulting from the $\pi/2$ pulses. With this pulse sequence, the shorter the T_1 value for a nucleus, the faster the decrease in amplitude of its resonance as t increases. 290 mM lecithin in CDCl_3 at 28°C : *a*, $t = 0.1$ s; *b*, $t = 1.0$ s. 230 mM lecithin in D_2O buffer at 52°C : *c*, $t = 0.1$ s; *d*, $t = 1.0$ s. Note the very short T_1 of the $\text{N}^+(\text{CH}_3)_3$ resonance relative to the $(\text{CH}_2)_n$ resonance in CDCl_3 , compared with the similar T_1 values of these two resonances in D_2O buffer (see also Table 1).

Table 1 T_1 Relaxation Times of Carbon Nuclei in Dipalmitoyl Lecithin in D_2O and CDCl_3

Carbon	T_1 (s)	
	D_2O (52°C)	CDCl_3 (28°C)
2	0.10	0.19
3	0.52	0.28
4-13	0.55	1.04
14	1.14	2.20
15	1.76	2.65
16	3.34	2.88
$\text{N}^+(\text{CH}_3)_3$	0.57	0.19

Palmitic acid carbons are numbered from the carboxyl group (1) to the terminal methyl (16).

spite of this, the methylene carbons at the terminal methyl end of the chains can be detected superimposed on the main envelope. Preliminary experiments suggest that the main envelope is broadened mainly by chemical shift differences rather than by any major differences in T_1 values for the same carbon nuclei in the sonicated and unsonicated states. No high resolution proton NMR spectrum could be obtained from the same unsonicated sample.

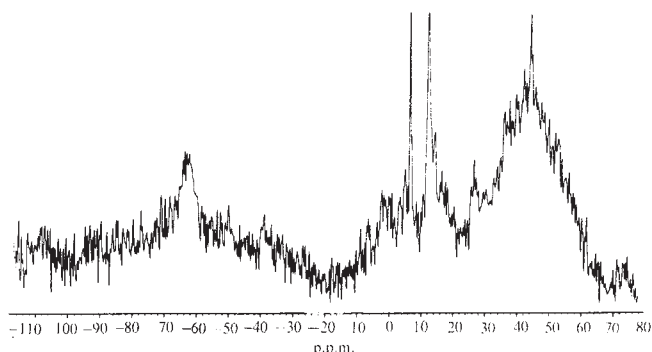


Fig. 3 The ^{13}C NMR spectrum of human erythrocyte membranes (10% w/w) in D_2O buffer at 28°C . The relatively sharp resonance at higher field (13 p.p.m.) is tentatively assigned to the $-\text{N}(\text{CH}_3)_3$ carbons of lecithin in the membrane; the sharp resonance at lower field (~ 7 p.p.m.) is possibly due to Trisious bound during the preparation of the membranes.

The observation of a spectrum from unsonicated lecithin suggested that biological membranes might yield ^{13}C spectra without sonication. The spectrum of 10% erythrocyte membranes in D_2O buffer at 28°C showed several broad envelopes of resonances, although the membrane suspension was extremely glutinous (Fig. 3). We will determine whether the broad envelopes of resonances result from many chemically shifted signals rather than from very short relaxation times, by selective ^{13}C enrichment of lipids biosynthetically incorporated into the membrane. If sharp resonances can be obtained from identified membrane components then ^{13}C NMR will provide detailed structural information about the membrane.

N. J. M. B. is seconded from Roche Products, Welwyn Garden City, Hertfordshire, A. G. L. holds Salters' Company and Kings College research fellowships, and Y. K. L. is a Beit Memorial Fellow.

J. C. METCALFE
N. J. M. BIRDSALL
J. FEENEY
A. G. LEE
Y. K. LEVINE
P. PARTINGTON

MRC Molecular Pharmacology Unit,
Medical School,
Hills Road,
Cambridge, CB2 2QD

Received May 25, 1971.

Release of Calcitonin from Thyroid Granules

THYROID parafollicular cells contain calcitonin in a granular form. Reduction in numbers of granules and vesicles occurs during induced calcitonin secretion *in vivo*¹, and from thyroid slices². Particulate fractions with the biological activity of calcitonin have been derived from homogenates of pig, calf and rat thyroid glands^{3,4}. We have prepared calcitonin containing particulate fractions from pig thyroid and have studied the properties of the isolated granules.

Sub-cellular fractions were prepared from pig thyroid glands by a modification of the method of Bauer and Teitelbaum⁴. Fresh glands were homogenized in 0.25 M sucrose with no additives. The effluent from the celite column was recentrifuged at 100,000g for 1 h, omitting the sucrose density gradient centrifugation, and was resuspended in barbital buffered saline (pH 7.2) containing 5 mg/ml. of bovine serum albumin. Calcitonin concentration was measured by radioimmunoassay. Tracer was prepared from synthetic porcine calcitonin and it was incubated with a guinea-pig antiporcine calcitonin serum prepared in this laboratory, at a final concentration of 1:15,000. The assay detects 50 pg of calcitonin in the 0.5 ml. incubation volume. Recoveries of calcitonin added to the granule fractions were 94–102%. Inhibition of binding by the fractions was parallel to the inhibition by standard, and there was close correlation between bioassay and immunoassay. The sub-cellular fractions were examined morphologically and biochemically. Granule fractions were comprised of dense granules and vesicles 50–200 nm diameter, some endoplasmic reticulum, ribosomes and occasional mitochondria. Beta glucuronidase⁵ and malic dehydrogenase⁶ concentrations relative to calcitonin were respectively 4.2 and 8.1 in the homogenate and 6.5 and 1.5 in the granule fractions.

Supernatant was separated from particulate calcitonin by centrifuging 1 ml. samples of the granule fraction at 0.8×10^6 g min. Greater centrifugal forces produced no further decrease in supernatant calcitonin concentration. Total and supernatant calcitonin were assayed in duplicate at two dilutions, and the fraction in particulate and supernatant pools was calculated.

Calcitonin was released into the supernatant with sonication, repeated freezing/thawing and with alterations of pH to 4.2 and 8.6 (Table 1). Filtration of granule suspensions through

Table 1 Calcitonin (%) in Supernatant after Various Stresses

Stress	Before	After
Sonication	33	85
Sonication	28	78
Freezing/thawing	19	48
Freezing/thawing	46	71
pH 3.5	46	69
pH 8.5	22	70

a 1.2 μm sartorius membrane filter did not remove particulate calcitonin. No spontaneous release of calcitonin into the supernatant occurred over 24 h at 0°C and 22°C , but at 37°C there was some release. This was not modified by the inclusion of 50 mM potassium chloride, 5 mM magnesium chloride, 5 mM calcium chloride or dibutyrylcyclic 3'5' AMP in the incubates (Table 2).

These results are consistent with the report that sonication increases calcitonin biological activity in particulate fractions of pig thyroid⁴. Freezing/thawing releases catecholamines from nerve storage granules⁷, alteration of pH releases catecholamines from adrenal⁸ and nerve granules⁷, and granules containing catecholamine are heat labile at physiological temperatures^{7,8}. Calcium and dibutyrylcyclic 3'5' AMP which release porcine calcitonin *in vivo*^{9,10} and from thyroid

¹ Birdsall, N. J. M., Lee, A. G., Levine, Y. K., and Metcalfe, J. C., *Biochim. Biophys. Acta*, **241**, 693 (1971).

² Hubbell, W. L., and McConnell, H. M., *J. Amer. Chem. Soc.*, **93**, 314 (1971).

³ Metcalfe, J. C., Seeman, P., and Burgen, A. S. V., *Mol. Pharmacol.*, **4**, 87 (1968).

⁴ Ernst, R. R., and Anderson, W. A., *Rev. Sci. Instrum.*, **37**, 93 (1966).

⁵ Vold, R. L., Waugh, J. S., Klein, M. P., and Phelps, D. E., *J. Chem. Phys.*, **48**, 3831 (1968).

⁶ Lippmaa, E., and Pehk, T., *Kem. Teollisuus*, **24**, 1001 (1967).

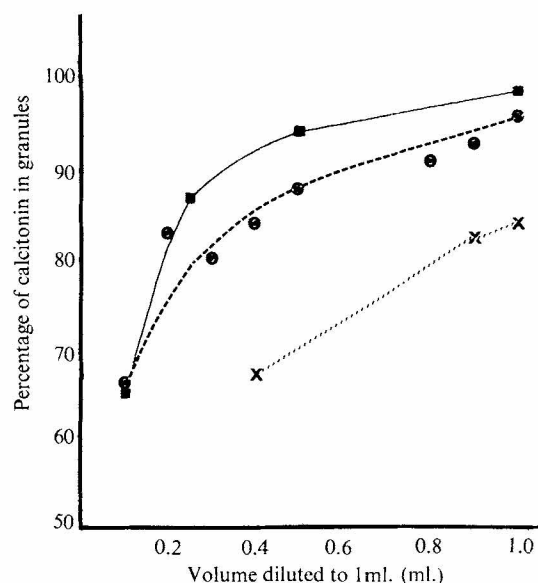


Fig. 1 Increasing volumes of granule suspension in buffer (0.025 M barbital, pH 7.2, 0.1 M saline, 5 mg % bovine serum albumin) were diluted in this buffer and incubated for 15 min at 4° C before separation of sedimentable from supernatant calcitonin. The percentage of calcitonin in the sedimentable fraction is shown as a function of dilution in three separate experiments.

slices¹¹ did not affect release from isolated granules; calcium has been shown to induce release of catecholamines from adrenal medullary granules in some electrolyte media¹².

As the granule suspensions were diluted in isotonic buffer release of calcitonin occurred (Fig. 1). For a ten-fold range of dilution, the percentage of particle-bound calcitonin decreased from 90–95% to 20–30%. This release was independent of bovine serum albumin concentration in the buffer over the range of 0.5–10 mg/ml.

Release of hormone on dilution in isotonic buffer occurs from histamine containing granules of mast cells¹³ and adrenal chromaffin granules in the presence of calcium¹⁴.

for synthetic porcine calcitonin, and Y. Euhara for the electron microscopy.

P. B. GREENBERG
T. J. MARTIN
R. A. MELICK

University of Melbourne,
Department of Medicine,
Royal Melbourne and Austin Hospitals,
Victoria

Received April 2, 1971.

- ¹ Matsuzawa, T., and Kurosumi, K., *Nature*, **213**, 927 (1967).
- ² Bussolati, G., Monga, G., Navone, R., and Gasparri, G., in *Calcitonin*, 240 (Heinemann, London, 1970).
- ³ Cooper, C. W., and Tashjian, A. H., *Endocrinology*, **79**, 819 (1966).
- ⁴ Bauer, W. C., and Teitelbaum, S. L., *Lab. Invest.*, **15**, 323 (1966).
- ⁵ Fishman, W. H., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. U.), 869 (Academic Press, New York, 1963).
- ⁶ Bergmeyer, H. U., and Bernt, E., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. U.), 757 (Academic Press, New York, 1963).
- ⁷ von Euler, U. S., and Lishajko, F., *Acta Physiol. Scand.*, **51**, 193 (1961).
- ⁸ Hillarp, N. A., *Acta Physiol. Scand.*, **43**, 292 (1958).
- ⁹ Care, A. D., Cooper, C. W., Duncan, T., and Orimo, H., *Endocrinology*, **83**, 161 (1968).
- ¹⁰ Care, A. D., Bates, R. F. L., Gitelman, H. J., *J. Endocrinol.*, **48**, 1 (1970).
- ¹¹ Bell, N. H., *J. Clin. Invest.*, **49**, 1368 (1970).
- ¹² Greenberg, R., and Sabelli, H. C., *Proc. Soc. Exp. Biol. Med.*, **121**, 1179 (1966).
- ¹³ Thon, I. L., and Uvnas, B., *Acta Physiol. Scand.*, **67**, 455 (1966).
- ¹⁴ Lishajko, F., *Acta Physiol. Scand.*, **79**, 575 (1970).
- ¹⁵ von Euler, U. S., in *Mechanisms of Release of Biogenic Amines* (edit. by von Euler, U. S., Rosell, R., and Uvnas, B.), 211 (Pergamon, London, 1966).

Significance of Surface Potential Measurements on Lipid Monolayers during Action of Phospholipase A on Lecithins

STUDIES on the action of enzymes on lipids and proteins of model membranes are aimed at identifying the relative position and orientation of the hydrophilic groups of the various membrane constituents^{1,2} so that models of the molecular organization and surface topography of membrane structures can be built.

Following discovery³ of the action of phospholipase A on films made of egg lecithin, Colacicco and Rapport described the phospholipase A action of two snake venoms on monolayers of two lecithins: phosphatidyl choline from egg and from beef heart⁴. The disappearance of lecithin and the appearance of lysolecithin in the film resulting from the action of phospholipase A are revealed by a decrease of surface potential: at neutral pH, the presence of fatty acid has little effect on the magnitude of the surface potential of lysolecithin⁴. It has been assumed^{1,4} that the fall in surface potential is proportional to the number of lecithin molecules transformed into lysolecithin, provided that the reaction products remain in the film. This is supported by two observations^{4,5}: first, thin-layer chromatography of film material showed that most of the lysolecithin was in the film; second, in conditions in which low protein concentrations (0.1 µg/ml. *Naja naja* venom) would not interfere with surface pressure and surface potential measurements, loss of reaction products would be accompanied by a considerable decrease in film pressure, which, however, was not seen^{4,5}. In contrast, when *Naja naja* venom (3.6 µg/ml.) was injected under high pressure films of ³²P-labelled yeast lecithin, surface radioactivity was lost rapidly and more than 80% lysolecithin was found in the

Table 2 Calcitonin (%) in Supernatant in Buffer with and without Addition of Ions and Dibutyrylcyclic 3'5' AMP

	0	1 h
Control	22	41
Calcium 5 mM	22	28
Magnesium 5 mM	29	42
Control	48	62
Calcium 5 mM	38	46
Potassium 50 mM		
Calcium 5 mM		
Magnesium 5 mM	41	50
Calcium 5 mM		
Magnesium 5 mM		
Potassium 50 mM	36	64
Control		
Dibutyrylcyclic 3'5' AMP 0.2 mM		
Dibutyrylcyclic 3'5' AMP 0.2 mM	19	31
Calcium 5 mM	—	46
Magnesium 5 mM		

Medium consisted of 0.025 M barbital, pH 7.2, 0.1 M saline, 5 mg % bovine serum albumin.

Release of calcitonin on dilution in isotonic buffer is consistent with the intra-cellular release of hormone from storage granules that von Euler has proposed for chromaffin granules¹⁵. Calcitonin release may be mediated at the cell membrane and intra-cellular stores repleted from the storage granules as the intracellular concentration falls.

This work was supported by the National Health and Medical Research Council. We thank Dr R. Neher (Ciba)

subphase, and Dawson argued⁶ that the reaction products left the film. This has thrown doubt on the assumption of Colacicco and Rapport⁷.

The conflict is traceable to basic differences in experimental conditions. Colacicco and Rapport used egg lecithin, the lysolecithin in which remains in the film at low pressure^{4,5}. Dawson used yeast lecithin⁶, the lysolecithin in which leaves the film readily at high pressures (near 30 dynes/cm). Some of the differences are examined here, and the surface potential measurement is shown to be an accurate method for determining the kinetics of the phospholipase A action on lecithin monolayers.

Yeast lecithin was obtained from Dr M. M. Rapport⁸. Dipalmitoyl lecithin and dimyristoyl lecithin were purchased respectively from Mann Research Laboratories, New York, and LaMotte Chemical Laboratories, Chestertown, Maryland. Preparations of egg lecithin and hydrogenated egg lecithin have already been described². Before use, lipids were purified by column chromatography on 'Unisil', from which the lecithins were eluted with chloroform-methanol mixtures. Fractions which were homogenous by thin-layer chromatography and gave a satisfactory analysis for phosphorus and esters were used⁹. Chain length and unsaturation of the egg lecithin and yeast lecithin used are known¹⁰. The technique for bringing lecithin and enzyme into contact has already been described; the rectangular trough was used⁴. The lecithin monolayer was spread at zero pressure on 0.04 M phosphate buffer, containing 58 μ M CaCl_2 (pH 7.0) 25° C, and 0.2 μ g/ml. *Naja naja* venom. The film was compressed rapidly (10 s) to the desired film pressure, and the changes in film pressure and surface potential were monitored as a function of time. Details of these and other techniques have been described^{1,2,4}. Surface pressure was determined by the Wilhelmy method; surface potential was measured with the use of a radioactive (²²⁶Ra) air electrode⁴. Unless it is otherwise specified, the rate of fall of surface potential is identified with the rate of hydrolysis of lecithin. The identity was established by the concomitant measurement of the relative quantities of substrate and product in collected lipid films⁴.

Decrease of surface potential, $-\Delta(\Delta V)$, in the first minute

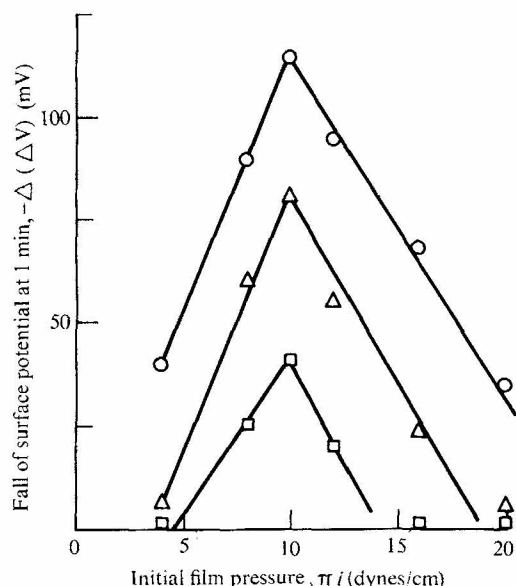


Fig. 1 Influence of initial pressure of lecithin film on decrease of surface potential, $-\Delta(\Delta V) \equiv$ hydrolysis, caused by action of phospholipase A in first minute. $\circ$, Yeast lecithin; Δ , dimyristoyl lecithin; $\square$, dipalmitoyl lecithin. Egg lecithin and hydrogenated egg lecithin are not shown: the curve of the first, with an optimum of 12 dynes/cm, was between the curves of yeast and dimyristoyl lecithins; the curve for the second was below that of dipalmitoyl lecithin. Temperature, 25° C.

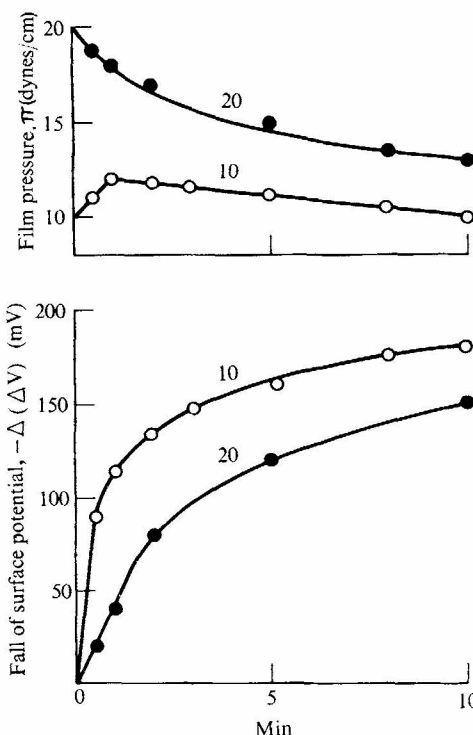


Fig. 2 Pressure-time (upper) and $\Delta(\Delta V)$ -time curves during the action of *Naja naja* venom on monolayers of yeast lecithin at two initial film pressures: 10 dynes/cm and 20 dynes/cm. The temperature was 25° C.

is related to the initial pressure of the lipid film (Fig. 1). The data show unequivocally, first, that all three lecithins—yeast, dimyristoyl and dipalmitoyl—displayed an optimal reaction at a film pressure of 10 dynes/cm; second, that with a given chain length (C_{16}), phospholipase A action is favoured by unsaturation (hydrolysis of monounsaturated yeast lecithin, which consists predominantly of dipalmitoleyl lecithin¹⁰, is markedly faster than that of fully saturated dipalmitoyl lecithin); and third, that of two saturated lecithins the one with shorter chains (dimyristoyl) is attacked more rapidly than the one with longer chain (dipalmitoyl).

The question of the conditions in which lysolecithin leaves the film is illustrated in Fig. 2 by the kinetic curves of the lipase action on monolayers of yeast lecithin. At the optimal film pressure of 10 dynes/cm, most of the lysolecithin remains in the film in the first 10 min as indicated by the little change in film pressure. Thin layer chromatography of films collected on a platinum gauze⁴ confirmed that 95% of lecithin was hydrolysed and more than 90% of it was in the film. At moderate film pressures, therefore, yeast lysolecithin does not leave the interface. But when the monolayer of yeast lecithin had an initial pressure of 20 dynes/cm, loss of film pressure and escape of lysolecithin from the film are so rapid that measurements of surface potentials past 2 or 3 min have to be regarded only as qualitative. Thin-layer chromatography of materials in the film and in the aqueous phase indicated that after 10 min only 70% of lecithin was hydrolysed and about 60% of it was in the hypophase. The rapid loss of pressure following the action of the venom on high pressure films of unsaturated lecithin is consistent with the pressure-time curves reported by Shah and Schulman in the action of *Naja naja* venom (0.05 μ g/ml.) on monolayers of highly unsaturated soybean lecithin⁵.

The ejection of lysolecithin from the film is far from quantitative, so that the measurement of surface radioactivity loss is inadequate for the description of enzyme kinetics; it is restricted to the high pressure films and highly unsaturated and very short chain lecithins (Fig. 2 and ref. 5). We therefore conclude that surface potential is the only method which

reveals instantaneously the transformation of lecithin into lysolecithin. Even in conditions in which lysolecithin is ejected from the film, the initial rate of fall of surface potential (for example in the first minute) truly reflects the enzyme kinetics. Concurrent determination of the reaction products either by surface radioactivity measurements and better by thin-layer chromatography and/or scintillation counting of aliquots from the hypophase² really establishes the validity of surface potential measurements. In contrast, measurement of surface radioactivity at low film pressure is useless if lysolecithin does not leave the film, and so also at high pressure in the known cases in which ejection of lysolecithin is not quantitative⁴⁻⁶.

Systematic studies can shed some light on the structural requirements of the lipid for hydrolysis of film lecithin by phospholipase A (as in Fig. 1) and ejection of lysolecithins, and in turn help to define the film pressure of biological membranes. Because the criteria used are not rigorous, the arguments in favour of low film pressure¹¹, for example 10 dynes/cm are as valid or liable as the arguments in favour of high film pressure, for example 30 dynes/cm or more¹². For given unsaturation and chain length of the membrane lecithins, ejection of lysolecithin after action of phospholipase A could become a criterion for establishing the film pressure of biological membranes.

This work was supported by the American Cancer Society and US National Institutes of Health Research.

GIUSEPPE COLACICCO *

Department of Biochemistry,
Albert Einstein College of Medicine,
Bronx,
New York 10461

Received January 20; revised April 19, 1971.

* Present address: Department of Pediatrics, Albert Einstein College of Medicine, Bronx, New York 10461.

- ¹ Colacicco, G., *J. Coll. Interface Sci.*, **29**, 345 (1969).
- ² Colacicco, G., *Lipids*, **5**, 636 (1970).
- ³ Hughes, A., *Biochem. J.*, **29**, 437 (1935).
- ⁴ Colacicco, G., and Rapport, M. M., *J. Lipid Res.*, **7**, 258 (1966).
- ⁵ Shah, D. O., and Schulman, J. H., *J. Coll. Interface Sci.*, **25**, 107 (1967).
- ⁶ Dawson, R. M. C., *Biochem. J.*, **98**, 35C (1966).
- ⁷ Pethica, B., in *Structural and Functional Aspects of Lipoproteins in Living Systems* (edit. by Tria, E., and Scanu, A. M.), 37 (Academic Press, New York, 1969).
- ⁸ Gottfried, E., and Rapport, M. M., *J. Biol. Chem.*, **237**, 329 (1966).
- ⁹ Colacicco, G., and Rapport, M. M., *J. Lipid Res.*, **8**, 513 (1967).
- ¹⁰ Shah, D. O., and Schulman, J. H., *J. Lipid Res.*, **6**, 341 (1965).
- ¹¹ Bar, R. S., Deamer, D. W., and Cornwell, D. G., *Science*, **153**, 1010 (1966).
- ¹² Jackson, E. M., in *Permeability and Function of Biological Membranes* (edit. by Bolis, L., Katchalsky, A., Keynes, R. D., Loewenstein, W. R., and Pethica, B. A.), 164 (North-Holland, Amsterdam, 1970).

Perceptual Fading of a Stabilized Cortical Image

AN image that is physically fixed on the retina rapidly seems to fade¹, and if the stabilization is good enough it never reappears^{2,3}. We have some evidence that this process of fading is not entirely retinal, but is partly central, for we have noticed that an image free to move over the retina, but "stabilized" on the visual cortex, also becomes less distinct.

Neurones of the visual cortex in cat and monkey respond best to line-shaped targets, black or white bars, of a particular orientation and often of a certain width^{4,5}. Many of them have quite large receptive fields on the retina and respond to the same sort of image anywhere within them.

There is psychophysical evidence that human visual systems also have this sort of organization, and that the neurones are central⁶⁻¹⁰. So presumably if we look in the middle of a

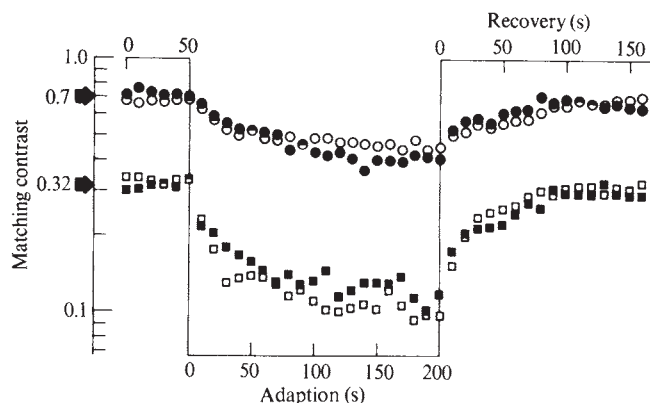


Fig. 1 Every 10 s the subject matched the contrast of the lower grating, shown on the ordinate, to that of the upper. Each symbol, open for one subject, solid for the other, is the mean of four settings made in separate experimental sessions. The average standard error of these means is only about 0.02 log units. For the circles the test grating on the upper screen had a contrast of 0.7, the same as that of the adapting pattern. For the squares the test grating had a contrast of 0.32. Contrast = $(I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$ where $I_{\max}$ $I_{\min}$ are the maximum and minimum luminances in the grating. ● ■, J. M.; ○ □, C. B.

pattern of black and white stripes (a grating), it is "stabilized" in our visual cortex. Within limits, wherever we move our gaze over the pattern, the same set of cortical neurones, specific for the orientation and width of the stripes, should be constantly stimulated. If our cortical cells show the same sort of attenuation of response that many sensory neurones suffer, then the activity aroused by such a pattern should gradually wane.

We have found that as one looks at a grating its apparent contrast declines and the pattern becomes less and less distinct. We arranged two oscilloscope screens, one above the other, each one subtending 4° in width and 3° in height. The gap between them was $24'$ and in the middle of it there was a luminous horizontal fixation bar. We generated on the screens vertical dark and light stripes, with sinusoidal luminance profile¹¹, and the experimenter could alter the spatial frequency (number of cycles of the grating per degree of visual angle) and contrast of the two patterns independently, and turn them on and off without changing their mean luminance, which was 4.8 cd m^{-2} for both screens.

Throughout the experiment the observer held his gaze on the fixation bar and his only task was to turn a logarithmic potentiometer to match the contrast of the pattern on the lower screen to that on the upper one. He pressed a button to print out his contrast setting. Every 10 s a test pattern of 5 cycles/degree appeared and the subject adjusted the contrast of the lower grating to match the upper one as quickly as possible. In between these settings the gratings on both screens were turned off. After the sixth such setting, the lower pattern disappeared but the upper one was replaced by an adapting grating of 5 cycles/degree at a contrast of 0.7. So now the subject was adapting the upper part of his visual field to the grating, while the lower part remained merely light adapted.

Every 10 s the original test grating appeared above and a comparison pattern below. The subject matched the contrasts quickly and the adapting situation was restored for a further 10 s before the next setting. After twenty such settings, with constant adaptation between each, the upper pattern was also extinguished between readings as we followed the time course of recovery.

The open and solid symbols in Fig. 1 show the results for two subjects. The contrast setting on the lower screen is plotted on the ordinate. For the circles, the test grating was identical in contrast (0.7) to the adapting pattern; for the squares the test grating had a contrast of only 0.32. In both

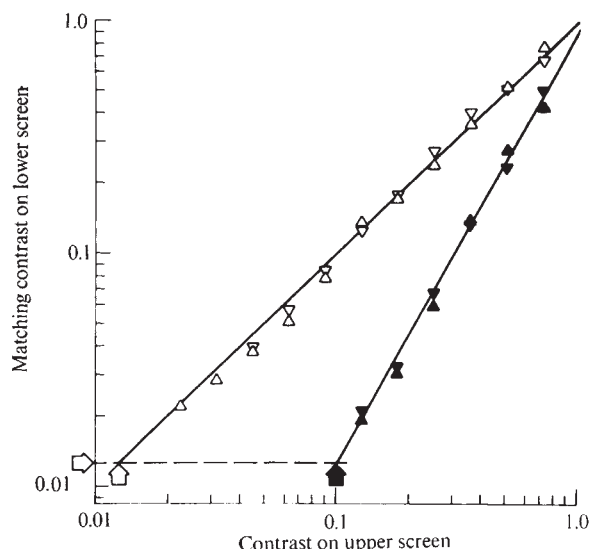


Fig. 2 The subject adapted constantly to a grating of 5 c/degree at a contrast of 0.7 on the upper screen. Every 10 s it was replaced by a test grating the contrast of which was varied from trial to trial. Each solid symbol is the mean of six settings of the contrast on the lower screen. A regression line (method of least squares) is fitted through them. The open symbols show the results for an identical series of contrast matches but with no prior adapting pattern on either screen. These points fit the expected line with a slope of +1. The open arrows indicate the threshold contrast before adapting ($N=6$; subject C. B.) and the solid arrow shows the elevated threshold on the upper screen after adaptation ($N=6$; C. B.). $\nabla \blacktriangledown$, J. M.; $\triangle \blacktriangle$, C. B.

cases there is a clear reduction in apparent contrast, with a time constant of about 30 s, but it is much greater for the lower contrast test grating.

Having seen that adapting to a high contrast grating can cause an apparent reduction in contrast that varies with the contrast of the test pattern, we performed the following experiment. The subject made a series of contrast matches for test gratings of 5 cycles/degree at various contrast levels: in between each setting both screens went blank. In Fig. 2 these unadapted settings are plotted as open symbols for the two subjects. The abscissa and ordinate are the contrasts on the upper and lower screens respectively. Not surprisingly, the open points lie near the expected function with a slope of +1.

Now the subject adapted constantly to a 5 cycles/degree grating at a contrast of 0.7 on the upper screen for 3 min, to allow the adaptation to build up to a maximum (Fig. 1). Then, as before, a test grating appeared on the upper screen with a comparison grating on the lower one and a match was made. At least 10 s of re-adaptation to the 0.7 contrast grating on the upper screen were allowed before the next pair appeared.

The contrast of the test grating was varied from trial to trial and the results are plotted as solid symbols in Fig. 2. Obviously, adapting to a contrast of 0.7 causes a reduction in apparent contrast at all test contrasts, but the magnitude of the effect is greatest at low contrast levels. Open arrows on the abscissa and ordinate mark the threshold contrast level for the stripes to be just distinguishable, before adaptation, and the solid arrow shows the new threshold contrast on the upper screen measured after adaptation. As Blakemore and Campbell described, the threshold is grossly elevated by prior adaptation¹⁰. Indeed elevation of threshold can be thought of as a special case of reduction in apparent contrast.

Fig. 2 shows an interesting point. Although at every contrast level the apparent contrast is reduced during adaptation, the slope of the function is steeper than +1. So the amplification or gain of the visual system for changes in contrast is actually increased by adaptation, although the absolute signal is smaller at all contrast levels. This increase in gain seems to be the first potentially beneficial consequence

of spatial adaptation that anyone has reported. If one looks at a grating, or presumably any other contour-containing pattern, one becomes more sensitive to changes in its contrast, like those effectively produced by fast eye movements, for example.

Finally, we have been able to use this new technique to measure the selectivity of human visual mechanisms for orientation and spatial frequency, at suprathreshold levels. If the adapting grating is replaced, not by a grating of identical orientation and spatial frequency, but by one that is markedly different, then the apparent contrast of the test grating is not reduced. This implies that the adapting and test gratings are handled by different neural channels. We shall be able to define the specificity of these channels and compare them with those revealed by threshold elevation⁸⁻¹⁰.

This work was supported by a grant to C. B. from the Medical Research Council.

COLIN BLAKEMORE

The Physiological Laboratory and

JAMES P. J. MUNCEY

ROSALIND M. RIDLEY

*The Psychological Laboratory,
University of Cambridge*

Received March 9; revised May 5, 1971.

- ¹ Riggs, L. A., Ratliff, F., Cornsweet, J. C., and Cornsweet, T. N., *J. Opt. Soc. Amer.*, **43**, 495 (1953).
- ² Barlow, H. B., *Quart. J. Exp. Psychol.*, **15**, 36 (1963).
- ³ Campbell, F. W., and Robson, J. G., *J. Physiol.*, **158**, 11P (1961).
- ⁴ Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **160**, 106 (1962).
- ⁵ Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **195**, 215 (1968).
- ⁶ MacKay, D. M., *Nature*, **180**, 849 (1957).
- ⁷ Andrews, D. P., *Nature*, **205**, 1218 (1965).
- ⁸ Gilinsky, A. S., *J. Opt. Soc. Amer.*, **58**, 13 (1968).
- ⁹ Pantle, A., and Sekuler, R., *Science*, **162**, 1146 (1968).
- ¹⁰ Blakemore, C., and Campbell, F. W., *J. Physiol.*, **203**, 237 (1969).
- ¹¹ Green, D. G., and Campbell, F. W., *J. Opt. Soc. Amer.*, **55**, 1154 (1965).

Iridescent Corneas in Fishes

DURING the course of underwater studies of fishes in the English Channel, Mediterranean and Indian Ocean, it was noticed that a coloured iridescent layer was often present on the inner surface of the cornea extending over the pupillary area (Fig. 1). Iridescence has been observed in a variety of animal tissues^{1,2} and is caused by regular multilayer structures stacked in a medium of different refractive index. Iridescent tissues already known in fish include the tapetum in which the principal material may be guanine¹ or collagen³ and the silvery iris and scales of teleost fish where the principal material is guanine⁴.

Walls³ noticed that in a few fishes including the gobies and particularly the Plectognaths (which include the Balistidae and Tetraodontidae) the corneal substantia propria exhibits a complex lamination with histologically peculiar intercalated layers. Iridescence is the rule among the groups Walls mentions and the iridescent layer can be seen by examination of the live fish to be situated at the inner surface of the cornea, so that it is reasonable to assume that the layers in the substantia propria are responsible for iridescence. In addition a similarly placed endothelial layer in the strongly iridescent cornea of the serranid fish *Nemanthias carberryi* possesses regular laminations that could well cause iridescence (personal communication from Locket).

Corneal iridescence has received scant, if any, attention in the scientific literature and should not be confused with the iridescent iris found in many fishes⁴. In the past corneal iridescence may have escaped notice since it is normally only

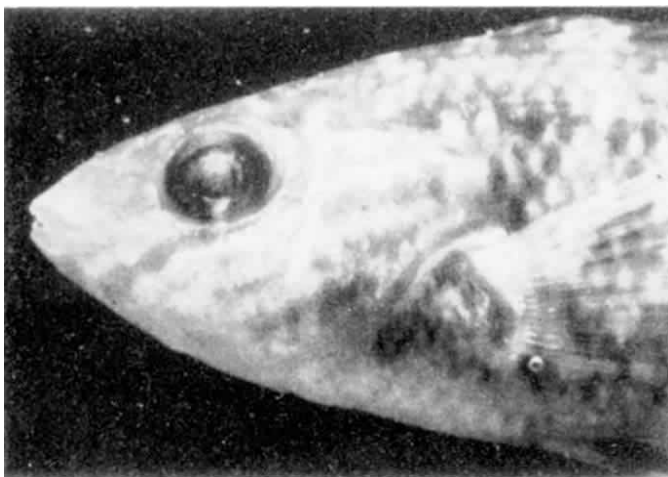


Fig. 1 *Crenilabrus melops* illuminated by electronic flash from above, showing corneal iridescence. The fish is alive, healthy and is in water. The cornea is green in colour, having a maximum reflectance in the region 520–550 nm.

visible when most of the illumination comes from above. This is the case in the natural environment⁵ but is less common in aquaria.

Corneal iridescence is common in members of the Tetraodontidae (puffer fishes), Balistidae (trigger fishes), Siganidae (rabbit fish), Scaridae (parrot fish), Gobiidae (gobies) and Labridae (wrasse). It has also been observed in the Cottidae and Serranidae. The colour varies from an unsaturated red in *Epinephelus merra* through yellow-green (*Arothron citrinella*) to a saturated blue-green (*Cottus bubalis*). There is, however, no obvious correlation between the colour of the iridescence and the colour of the water in which the fish lives.

Fishes with iridescent corneas often, but not always, possess yellow filters⁶ at some point in the optical pathway. Thus of the fishes with iridescent corneas mentioned here, *Arothron citrinella*, *Crenilabrus melops*, *Cottus bubalis* and *Balistapus undulatus* have yellow corneas; *Xanothon bipallidus* and *Callyodon viridifucatus* have yellow lenses. No yellow coloration has been observed in the lens or cornea of *Epinephelus merra* or *Siganus stellatus*. The aqueous or vitreous humours were clear except in the case of a yellow vitreous humour in *Siganus rostratus*. The cornea of *Nemanthias carberryi* was clear but the lens was not examined.

The data that follow were obtained from fishes collected by the use of explosives at the Royal Society's Research Station on Aldabra in the Indian Ocean. Immediately after the charge was detonated, three divers entered the water; one to collect fish, one to watch for sharks and one to study iridescence of the collected fishes.

The fish, killed by the explosion one to two minutes previously, was clamped to a plastic writing board with an inclinometer attached at the lower left hand corner. The inclinometer consisted of a glass sphere open at the bottom and marked with lines of latitude and longitude. A metal rod was fastened inside the sphere in such a way that its tip marked the geometric centre of the sphere. Underwater the sphere filled with water; a small air bubble was then introduced and this indicated the vertical. The direction of view was obtained by noting the locus on the surface that aligned with the centre of the sphere. Observations were made as near noon as possible at 15–25 m. depth. In this way the direction of maximum downwelling radiance corresponded with the vertical within the limits of experimental error.

The apparatus was held level with the eye so that the anterior/posterior axis and the axis between the eyes was horizontal. In *Callyodon*, *Xanothon* and *Balistapus* corneal iridescence was visible in this position. In *Nemanthias* the fish had to be slightly below the level of the observer's eye.

Table 1 The Angles of Pitch and Roll through which Corneal Iridescence is Visible

	Pitch	Roll
(A) <i>Nemanthias carberryi</i>	–25° +25°	–0° +66°
(B) <i>Balistapus undulatus</i>	–61° +54°	–49° +59°
(C) <i>Xanothon bipallidus</i>	–51° +32°	–10° +68°
(D) <i>Callyodon viridifucatus</i>	–66° +44°	–25° +66°

The angles are measured from the vertical. Negative pitch is when the head is in the up position; negative roll is when the back of the fish is tilted away from the observer.

The apparatus with fish attached was then rotated around the anterior/posterior axis (roll) and then around the axis joining the eyes (pitch). The angles of pitch and roll through which iridescence was visible are listed in Table 1. When iridescence is visible under natural conditions the entire corneal area is affected. When a small artificial light source is used as in Fig. 1, the sector of the cornea shaded from the light does not appear iridescent. The fishes were then viewed from different directions, keeping the anterior/posterior axis and the inter-eye axis horizontal. When viewed from below and somewhat from in front, no iridescence was visible (Fig. 2).

It is now well established that the silvery iridescent scales and iris of fish act as a camouflage device underwater⁴ and it might seem natural to ascribe the same function to corneal iridescence. To the diver, however, corneal iridescence often shows up extremely brightly underwater and is rarely the same colour as the head; for instance, corneal iridescence in *Nemanthias* is blue-green whilst the head and body is golden orange. Furthermore, the cornea is not iridescent in silvery sided pelagic fishes where the black pupil may be the only visible part of a fish swimming in open water⁴.

It is too early to speculate on the role of the yellow optical filters and the iridescent cornea on selective spectral absorption of light from different directions, but all the fishes so far known

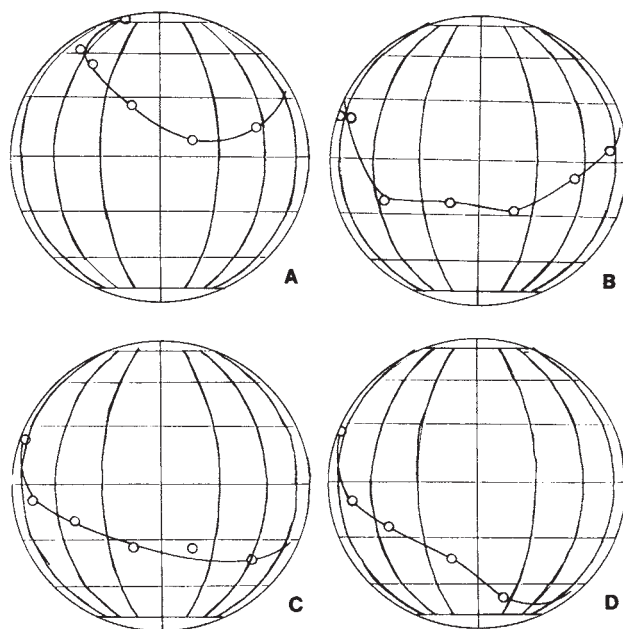


Fig. 2 Direction from which corneal iridescence is visible. The diagram represents the curved surface of a cornea oriented in the same way as in the fish in Fig. 1. The angle of view is indicated by the locus on the sphere's surface which aligns with the centre of the sphere. The line joining the circles indicates the direction of view at which the entire surface of the cornea appears iridescent. Iridescence ceases to be visible when the direction of view is from below the line. A, *Nemanthias carberryi*; B, *Balistapus undulatus* (trigger fish); C, *Xanothon bipallidus* (parrot fish); D, *Callyodon viridifucatus* (parrot fish).

to possess this iridescence live and feed near the bottom and it may be that the iridescence acts as a sophisticated sunshade designed to reduce by reflexion the bright light arriving from the upper hemisphere, whilst allowing the much less bright light entering from the direction of the sea bed to pass without significant loss.

I thank the staff of the Royal Society Research Station and my co-divers, Mr J. A. Stevenson, Dr W. R. A. Muntz and Mr A. J. Young for their support and help.

J. N. LYTGOE

MRC Vision Unit,
University of Sussex

Received June 4, 1971.

- ¹ Denton, E. J., and Land, M. F., *J. Physiol. Lond.*, **191**, 23P (1967).
- ² Bernard, G. D., and Miller, W. H., *Invest. Ophthalmol.*, **7**, 416 (1968).
- ³ Walls, G. L., *The Vertebrate Eye* (Hafner, New York and London, 1963).
- ⁴ Denton, E. J., and Nicol, J. A. C., *J. Marine Biol. Assoc.*, **46**, 685 (1966).
- ⁵ Jerlov, N. G., *Optical Oceanography* (Elsevier, New York, London, Amsterdam, 1968).
- ⁶ Moreland, J. D., and Lythgoe, J. N., *Vision Res.*, **8**, 1377 (1968).

Titration of Oral Nicotine Intake with Smoking Behaviour in Monkeys

SEVERAL studies have indicated that nicotine is an incentive in smoking. Johnston¹ found that heavy smokers reported a pleasurable sensation when given nicotine, whereas non-smokers reported an unpleasant effect. Lucchesi *et al.*² showed that intravenous administration of nicotine diminished the number of cigarettes smoked by heavy smokers. Jarvik *et al.*³ found that oral nicotine administration produced a very small but significant decrease in the number of cigarettes smoked but because humans were used, they³ were not able to use large doses of nicotine which might have produced larger decrements in smoking. Previous work in this laboratory with monkeys trained to puff cigarette smoke showed that mecamlamine, a nicotinic-blocking agent, reduced their smoking⁴, and the work reported here was conducted to establish more definitely that orally administered nicotine could substitute for nicotine derived from smoking.

Four mature rhesus monkeys were trained to puff cigarette smoke. Only one (Phoebe) had served as a subject in the previous study with mecamlamine. Puffing behaviour was instilled initially by making the monkeys suck on a tube in order to drink. The mouthpiece of a cigarette smoking apparatus⁵ was then substituted for the water tube. The smoking apparatus allowed a monkey to smoke lit cigarettes by automatically lighting each cigarette, spacing the cigarettes over time, and sensing changes in pressure as the monkey puffed. When the monkeys had learned to puff, they were so trained that they had to puff but were allowed to choose between smoke and air⁶. This procedure was developed to reduce the large variability from subject to subject inherent in free puffing.

A smoking apparatus was attached to a 'Plexiglas' panel covering a 36 cm square opening in the door of each monkey's home cage. Two tubes, one delivering cigarette smoke and a "dummy" tube with access only to air, were also mounted on the panel. A water solenoid was mounted between the smoke and air tubes. A puff on either the smoke or air tubes preset the solenoid and would release a small amount of water when the monkey touched the solenoid spout. A light mounted above the solenoid signalled the availability of water. A fixed ratio (FR) contingency was added, so that a monkey could be required to puff a specific number of times to obtain water.

The monkeys received all their water during a daily 4 h puffing session. Thirty cigarettes were loaded into the smoking apparatus each morning and the test was started at 1030 h. A new cigarette was automatically lit every 7.5 min. The positions of the smoke and air tubes were interchanged each morning so that side preferences would not develop. Initially, water could be won with a single puff on either the smoke or air tube. The number of puffs for a reward of water was then increased to five, ten, twenty and finally to thirty. The FR 30 schedule was then maintained. Puffs were recorded on Sodeco counters.

All four monkeys preferred smoke to air; that is, although they could get water by puffing on either the smoke or the air tubes, they reliably took more puffs on the smoke tube than on the air tube. When puffing rates on the FR 30 schedule stabilized, the nicotine experiment was begun (except for the monkey used in the previous mecamlamine experiment⁴; the present experiment was started 2 months after completion of the previous experiment).

Various quantities (50 mg to 200 mg) of nicotine tartrate were dissolved in the water which each monkey obtained through puffing. The amount of nicotine on a given day was always dissolved in 700 ml. of water although the monkeys would usually consume only 300–400 ml. during a puffing session. Increasing dosages of nicotine were self-administered in this way with at least 4 days between successive nicotine trials. Each dose was repeated two or three times before starting a higher dose.

The results obtained from such oral nicotine administration are shown in Table 1. The data on days immediately preceding

Table 1 Changes in Smoke Preference induced by Oral Nicotine

	Phoebe		Alex		Ivan		Dot	
Dose (mg)	Total puffs	% pref.	Total puffs	% pref.	Total puffs	% pref.	Total puffs	% pref.
Control	6,103	88.7	4,844	86.4	2,786	87.2	4,322	91.1
50	6,509	87.5			2,594	87.0	3,666	88.3
75	6,466	91.0	5,191	90.0	3,465	87.0	4,437	86.0
100	6,440	92.0			3,453	75.0	4,754	81.5
125	5,784	64.1*	4,798	91.9	2,589	41.7*	4,308	14.8*
175			4,973	75.0				
225			4,623	56.7*				

* Significantly less than control at $P < 0.05$.

oral nicotine days were used as control data in paired *t* tests. For each monkey, doses of oral nicotine were found which significantly decreased and in some cases reversed the smoke-air preference without significantly affecting the overall puffing rate.

One additional drug trial was conducted with one of the monkeys. Because previous work had shown that mecamlamine inhibited monkeys' smoking, it was of interest to determine whether mecamlamine would antagonize the inhibition of smoking by orally administered nicotine, that is, whether the combination would depress smoking behaviour less than either treatment alone. For this purpose, the monkey used in the previous mecamlamine experiment as well as the more recent oral nicotine experiment was used again. A dose of 125 mg of nicotine tartrate was administered orally as already described. In addition, a dose of 0.8 mg/kg of mecamlamine was given intramuscularly 15 min before the beginning of the same puffing session. This combination was repeated twice. Thereafter, two administrations of the mecamlamine alone were conducted. The results are shown in Table 2. All three treatments—oral nicotine alone, mecamlamine alone and the combination—produced significant inhibition of smoking behaviour. Only mecamlamine alone and the combination significantly decreased overall puffing. Most surprising, however, was the finding that the inhibition of smoking produced by the combination was significantly greater ($P < 0.05$) than that produced by either drug treatment alone.

Table 2 Changes in Smoke Preference of Phoebe induced by Oral Nicotine (125 mg) and/or Mecamylamine (0.8 mg/kg)

	Total puff	% pref.
Control	6,055	88.2
Nicotine	5,784	64.1 *
Mecamylamine	4,842 *	65.5 *
Nicotine + mecamylamine	3,358 *	33.5 *

* Significantly less than control at $P < 0.05$.

These results raise interesting questions about the role of nicotine in smoking. If oral nicotine depressed smoking by providing a greater than optimal level of nicotine, such that additional nicotine derived from smoking was aversive, then mecamylamine should have antagonized rather than potentiated this effect. However, different central actions of nicotine may have been responsible. Perhaps the aversiveness produced by the large oral nicotine doses was a function of nicotinic blockade rather than of activation⁷. Blockage of nicotinic activating, presumably rewarding, actions of nicotine by mecamylamine would then allow non-rewarding blocking actions to predominate. Clinical studies are being conducted in this laboratory to evaluate the therapeutic significance of this hypothesis.

This work was supported by grants from the US National Institute of Mental Health and the American Cancer Society.

S. D. GLICK*
B. ZIMMERBERG
M. E. JARVIK

Department of Pharmacology and Psychiatry,
Albert Einstein College of Medicine,
1300 Morris Park Avenue,
Bronx, New York 10461

Received June 21, 1971.

* Present address: Department of Pharmacology, Mount Sinai School of Medicine, New York, NY, 10029.

- Johnston, L. M., *Lancet*, ii, 742 (1942).
- Lucchesi, B. R., Schuster, C. R., and Emley, G. S., *Clin. Pharmacol. Ther.*, **8**, 789 (1967).
- Jarvik, M. E., Glick, S. D., and Nakamura, R. K., *Clin. Pharmacol. Ther.*, **11**, 574 (1970).
- Glick, S. D., Jarvik, M. E., and Nakamura, R. K., *Nature*, **227**, 969 (1970).
- Pybus, R., Goldfarb, T., and Jarvik, M. E., *Exp. Anal. Behav.*, **12**, 88 (1969).
- Glick, S. D., Canfield, J. L., and Jarvik, M. E., *Psychol. Rep.*, **26**, 707 (1970).
- Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics*, 585 (Macmillan, London, 1965).

Effects of Cyprenorphine Hydrochloride on Sensory Reinforcement in the Rat

IN a previous study¹ of the morphine antagonist cyprenorphine hydrochloride (M 285), we examined the possible hallucinogenic effects of this analgesic compound on the light-reinforced behaviour of rats. Control animals receiving saline injections showed typically increased response rates during test sessions in which response-contingent light (RCL) was available, whereas M 285 (0.3 mg/kg) depressed this light reinforcement (LR) effect. The drug had no effect on operant or non-reinforced responding, and so we postulated that it had its principal effect in relation to the relative significance of the stimulation to the animal. More specifically, this drug (in common with other hallucinogenic agents) may raise the arousal level of an

animal that is relatively deprived visually, so that it needs very little further stimulation.

We report now a further test of the hypothesis that M 285 has a direct sensory effect. A response-contingent light offset (RCLO) situation involves sensory reinforcement, just as in the RCL onset situation, but the sensory conditions are reversed. If the visual stimulation is hallucinogenically intensified by injection of M 285, then the drug group should respond more than the saline group in order to turn the light off for a short period.

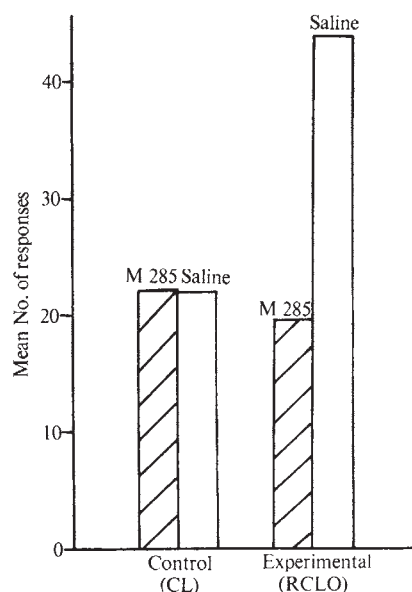


Fig. 1 Effect of M 285 (0.3 mg/kg) on reinforced (RCLO) and non-reinforced (CL) responding in rats. Response rates were recorded from a 60 min test session on day 2. All four groups ($n=9$) were matched for mean baseline response rates in a 30 min CL session on day 1.

Thirty-six female hooded rats, 80–90 days old, were tested in the six sound-insulated, light-proof Skinner boxes described in the previous study¹. Each rat was placed in a response box with the end ('Perspex') wall dimly illuminated constantly throughout an initial 30 min baseline session. A response (lever press) produced no change in the stimulus condition. After this "constant light" (CL) period, the thirty-six rats were matched in four groups of nine on the basis of their average operant response rate during this first CL session. On the following day, two groups received 0.3 mg/kg of M 285 intraperitoneally in 0.9% saline solution. The other two groups were injected with saline. Ten minutes after injection the rats were placed in the same response boxes with constant dim illumination. One drug group and one saline group were run in the experimental condition, in which a lever press switched off the light for as long as the lever was depressed (RCLO). The two control groups (drug and saline) were run under constant light (CL) as on day 1, and their responses produced no change in the stimulus conditions. The control (CL) and experimental (RCLO) sessions on day 2 lasted for 60 min.

Fig. 1 shows the response rates of the four groups during the test sessions on day 2. In the experimental (RCLO) condition there is a very significant difference between the drug and saline animals ($t=4.9$; d.f. = 16; $P < 0.001$). The saline group shows a typical sensory reinforcement effect, since the mean response rate was twice that of the control (CL) group. Response rates of experimental animals injected with M 285, however, were no different from those of the control groups.

We have thus replicated, in a different experimental situation (RCLO), the finding that M 285 (at a dosage of 0.3 mg/kg) selectively depresses a sensory reinforcement effect without affecting non-reinforced operant responding. This result, however, contradicts the expected increase in responding of the experimental M 285 group, predicted from the hypothesis that visual stimulation is hallucinogenically intensified. A more likely explanation is that the sensory change is the critical stimulating and reinforcing factor, irrespective of whether this change involves onset or offset². Thus, RC changes in sensory stimulation would be less rewarding for animals whose arousal level has been raised by M 285 than for saline animals.

We thank Reckitt and Colman for supplies of M 285, and Dr A. L. A. Boura for advice and assistance. The work was supported by a grant from the Medical Research Council.

G. LOWE
D. I. WILLIAMS

Department of Psychology,
University of Hull

Received April 5, 1971.

¹ Lowe, G., and Williams, D. I., *Nature*, **224**, 1226 (1969).

² Williams, D. I., and Lowe, G., *Brit. J. Psychol.*, **61**, 379 (1970).

Characterization of the Principal Steroidal Saponins of the Starfish *Marthasterias glacialis*: Structures of the Aglycones

STEROID glycosides with surface active properties have been isolated from extracts of the starfish, *Marthasterias glacialis*, by gel filtration and ion exchange chromatography^{1,2}. These saponins induce avoidance reactions in the mollusc, *Buccinum undatum*, and may play a part in reducing predation by the starfish on this mollusc and other marine organisms. Mackie³ has discussed the possible significance of this type of interaction in relation to pollution studies. Partial characterization of one of the major glycosides showed it to be a mixed conjugate of a Δ^{24} -23-ketocholestane derivative². In this communication we discuss the composition of the glycoside mixture and evidence for the nuclear structure of the aglycone.

Crude starfish extracts were hydrolysed with hydrochloric acid and the water-insoluble aglycone was purified by chromatography on silica gel. Although homogeneous by thin-layer chromatography in several solvent systems, the aglycone was resolved into three components (ratio approximately 1:5:4, varying slightly with different batches of animals) by gas chromatography. The major components were then separated by preparative layer chromatography using continuous development in benzene-ether (24:1 v/v). The more polar of these, now called marthasterone, contained the Δ^{24} -23-ketone system previously characterized² ($\lambda_{\max}$ 240 nm; $\nu_{\max}$ 1,690 cm^{-1}), while the other component exhibited no selective absorption in the ultraviolet and its carbonyl stretching frequency appeared at 1,715 cm^{-1} . This, together with its nuclear magnetic resonance (NMR) spectrum, suggested that it was the 24,25-dihydroderivative of marthasterone, and the relationship was confirmed by selective hydrogenation (Pd/C, 1 atmosphere, 1 h) of the side-chain double bond of marthasterone, which yielded the other major component. Further structural work was then carried out on this material, melting point 167°–169° C, now called dihydromarthasterone.

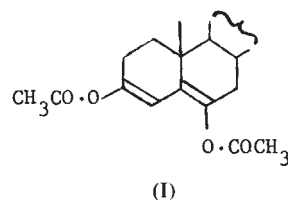
Dihydromarthasterone ($\text{C}_{27}\text{H}_{44}\text{O}_3$, M^+ 416.3289 (required 416.3290)) showed a mass spectral fragmentation pattern analogous to that of marthasterone, with the major cleavage (at C_{20} – C_{22}) involving loss of the six-carbon fragment containing the ketone function (m/e 316, 301, 299, 298, 283, 265).

Previous work indicated that the nuclear substituents of the aglycone consisted of a double bond and two hydroxyl groups², and spectral data established conclusively that the trisubstituted nuclear double bond was not attacked in the above conditions. Prolonged catalytic hydrogenation (50 h) was required to reduce the nuclear double bond, as shown by the disappearance of the olefinic proton signal at τ 4.68. In the resulting tetrahydromarthasterone, the C_{19} angular methyl group showed a large upfield shift (10 Hz), while the C_{18} angular methyl had moved downfield (2 Hz). Zürcher's rules⁴ indicated that this was in accordance only with the presence of a $\Delta^9(11)$ -olefinic bond in the substrate. Other trisubstituted double bonds produced markedly different effects on the positions of the angular methyl group resonance frequencies. (Hydrogenation of the side-chain double bond had no significant effect on these resonances.)

Epoxidation of the nuclear double bond, using monoperphthalic acid in ether, was accompanied by loss of the olefinic signal at τ 4.71 and the appearance of a broad one-proton doublet at τ 6.83 ($J=6$ Hz). In addition, downfield movements of the C_{19} (14 Hz) and C_{18} (5 Hz) protons were noted. Similar changes occurred in the positions of the angular methyl proton signals following the epoxidation of a number of model $\Delta^9(11)$ -compounds ($\Delta^9(11)$ -tigogenin acetate, $\Delta^9(11)$ -pregnanolone acetate, and so on). There is thus strong evidence that the nuclear double bond of marthasterone occupies the 9,11-position.

The locations of the two nuclear hydroxyl groups were next investigated. Oxidation of marthasterone with chromic acid in acetone gave a triketone ($\lambda_{\max}$ 239 nm, $\epsilon=10,120$; $\nu_{\max}$ (KBr) 1,715 and 1,680 cm^{-1}). Its NMR spectrum showed downfield shifts (C_{19} , 15 Hz; C_{18} , 5 Hz) for the angular methyl groups, and the olefinic proton at C_{11} had also moved 16 Hz to lower field. This indicates that the keto-groups are relatively close to the C_{19} angular methyl group and the $\Delta^9(11)$ -bond, but more remote from the C_{18} angular methyl group. Chemical shifts for the angular methyl protons calculated from literature values⁴ were in good agreement for 3 and 6-keto groups, as were the observed values for the triketones obtained by similar oxidation of dihydro and tetrahydromarthasterone. Further evidence for this assignment was obtained by oxidation of 3 β ,6 α -dihydroxy-5 α -cholestane to 5 α -cholestane-3,6-dione. This was accompanied by downfield shifts of 13 and 4 Hz, respectively, for the 19 and 18-protons. Other positions for the two nuclear oxygen functions were excluded by reference to literature compilations^{4,5}.

Attempts to confirm the 3,6-relationship of the new keto-groups by dehydrogenation to the conjugated Δ^4 -3,6-dione system using selenium dioxide were unsuccessful. Treatment of the triketone with isopropenyl acetate, however, produced a diacetate mixture having $\lambda_{\max}$ 244.5 nm ($\epsilon=3,430$). The corresponding product mixture from 5 α -cholestane-3,6-dione had $\lambda_{\max}$ 245 nm ($\epsilon=3,450$). This absorption corresponds to that of an heteroannular diene chromophore of type (I). The

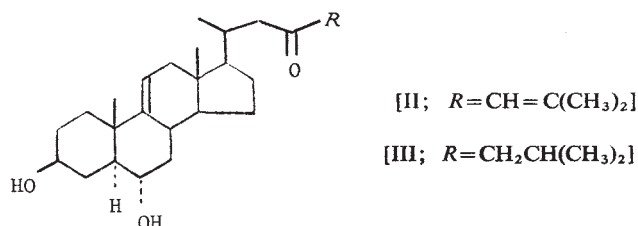


acetylated mixtures from the two compounds were shown by gas-liquid chromatography to consist of similar mixtures of components, resulting from the various enolization pathways open to the diketones. The major products appear to be the $\Delta^{3,5}$ and $\Delta^{2,5}$ -dien-3,6-diol diacetates.

The broad nature of the C_3 and C_6 proton signals in the original aglycones and their diacetates indicate that they are

both axial; hence the hydroxyl groups are equatorial, and occupy the 3 β and 6 α positions.

NMR spectral data on this series of compounds accord with the A/B-*trans*-ring junction, rather than the A/B-*cis*-junction, and hence the structures proposed for the major aglycones present in the *Marthasterias* saponins are 3 β ,6 α -dihydroxy-5 α -cholesta-9(11),24-dien-23-one (marthasterone, II) and its 24,25-dihydro-derivative (III).



Two structural features of these molecules are of biogenetic interest. First, the keto-groups at position 23 are unique, oxygenation at this position in the side chain having only previously been encountered in nature in the case of 23 ξ -hydroxylanosterol. This was isolated from the common fungus, *Scleroderma aurantium*, by Entwistle and Pratt^{6,7}. The nuclear hydroxylation pattern occurs in the cactus sterols, peniocerol⁸ and macedougallin^{9,10}. Second, starfish sterols are characterized by the presence of a Δ^7 -bond¹¹, as are those of sea cucumbers. The latter, however, also produce toxic steroidal saponins (the holothurinogenins) possessing a $\Delta^{7,9(11)}$ -diene system^{12,13}, and $\Delta^9(11)$ -derivatives¹⁴.

Recent work^{15,16} shows that echinoderm sterols are partly dietary in origin and partly biosynthesized by the animals. Incubation of starfish with 2-¹⁴C-mevalonic acid gave heavily labelled lanosterol, although cholest-7-enol, the principal sterol component, was only weakly radioactive.

Synthetic work to confirm the structures proposed is in progress in this laboratory, as well as further work on the location of the conjugating groups in the original saponins, and characterization of the minor component.

A. B. TURNER
D. S. H. SMITH

Department of Chemistry,
University of Aberdeen,
Aberdeen AB9 2UE

A. M. MACKIE

Natural Environment Research Council,
Fisheries Biochemical Research Unit,
University of Aberdeen,
Aberdeen AB1 3RA

Received May 26, 1971.

- ¹ Mackie, A. M., Lasker, R., and Grant, P. T., *Comp. Biochem. Physiol.*, **26**, 415 (1968).
- ² Mackie, A. M., and Turner, A. B., *Biochem. J.*, **117**, 543 (1970).
- ³ Mackie, A. M., *J. Exp. Marine Biol. Ecol.*, **5**, 63 (1970).
- ⁴ Zürcher, R. F., *Helv. Chim. Acta*, **44**, 1380 (1961).
- ⁵ Zürcher, R. F., *Helv. Chim. Acta*, **46**, 2054 (1963).
- ⁶ Entwistle, N., and Pratt, A. D., *Tetrahedron*, **24**, 3949 (1968).
- ⁷ van Lier, J. E., and Smith, L. L., *J. Org. Chem.*, **36**, 1007 (1971).
- ⁸ Djerassi, C., Murray, R. D. H., and Villotti, R., *J. Chem. Soc.*, 1160 (1965).
- ⁹ Djerassi, C., Knight, J. C., and Williams, D. I., *J. Amer. Chem. Soc.*, **85**, 835 (1963).
- ¹⁰ Djerassi, C., Knight, J. C., and Williams, D. I., *J. Amer. Chem. Soc.*, **88**, 790 (1966).
- ¹¹ Gupta, K. C., and Scheuer, P. J., *Tetrahedron*, **24**, 5831 (1968).

- ¹² Tursch, B., Guimaraes, I. S. S., Gilbert, B., Aplin, R. T., and Djerassi, C., *Tetrahedron*, **23**, 761 (1967).
- ¹³ Roller, P., Djerassi, C., Cloetens, R., and Tursch, B., *J. Amer. Chem. Soc.*, **91**, 4918 (1969).
- ¹⁴ Chanley, J. D., and Rossi, C., *Tetrahedron*, **25**, 1897, 1191 (1969).
- ¹⁵ Smith, A. G., and Goad, L. J., *FEBS Lett.*, **12**, 233 (1971).
- ¹⁶ Goad, L. J., *Abstr. Roy. Soc.*, March 25 (1971).

Lead Accumulation by Wild Oats (*Avena fatua*) in a Contaminated Area

THE input of lead into the environment has increased during the last two decades¹⁻⁴, especially with the development of lead-containing gasolines². The body-burden of lead is now much greater in man than in his modern ancestors⁵ and as one source of lead is food, its acquisition, mobility and effects in plants and animals, as well as its partitioning among the air, soil, water and biotic fractions, must be understood.

The Benicia-Vallejo area, 25 miles north-east of San Francisco, is a unique site for such studies because it has been continuously contaminated with lead from a nearby smelter for approximately 70 yr⁶. The area includes both agricultural and urban uses of land. Between early 1969 and early 1970 thirteen Appaloosa horses died while grazing in a winter pasture in the area and their deaths were correlated with a high body-burden of lead. Since then we have carried out an intensive sampling and analysis of native vegetation.

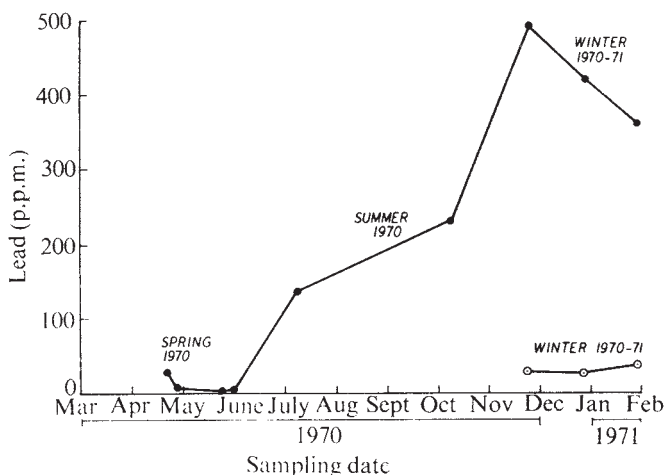


Fig. 1 The concentration of lead (p.p.m. of dry weight) in the above-ground portion of *Avena fatua* as a function of sampling date. ●, 1969-70 growing season; ○, 1970-71 growing season.

The aerial parts of various plants were analysed for lead by atomic absorption spectrophotometry⁷. Tests on two dates indicated no detectable difference in lead concentrations between washed and unwashed samples. Fig. 1 summarizes the analysis of *Avena fatua* samples. Initially, during the period of rapid growth and heading out (April-June), the lead concentrations decreased, but then increased substantially during ripening until autumn, when the plants were completely air-dry. The increase continued, accelerating after heavy rain in October and November when the new season's growth began, and reached a peak in December. Late November to late January was also a period of heavy rain. Lead contamination in the new growth was similar to that observed in the early samples of the 1970 season.

The shape of the curve in the early months of the year may reflect growth dilution. The plants are growing and are con-

stantly absorbing lead from either the soil or the air, and a decrease in concentration might be expected. As the plants senesce and desiccate the lead concentration would increase because, although input may remain constant, the mass of the plants, is no longer increasing. Later in the season, however, when the plants and soil are dry, the transfer of lead from the soil to the shoots would presumably cease. The observed continuation of the accumulation of lead probably, therefore, derives from atmospheric inputs.

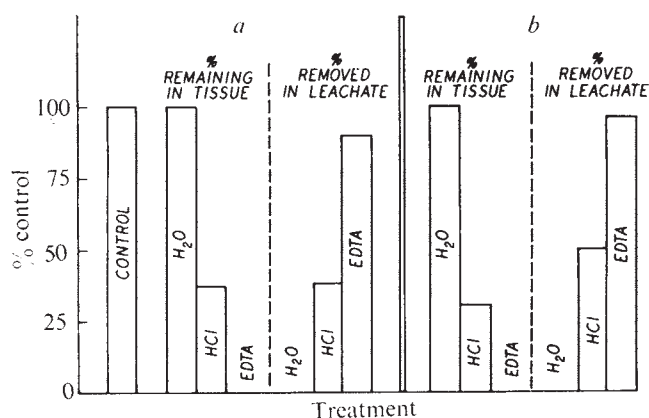


Fig. 2 Leaching for (a) 4 and (b) 24 h of ground plant material by various solutions. The results are expressed as percentage of lead in control, represented by untreated straw. Treatments consisted of H₂O, 0.01 N HCl and 0.01 M NH₄-EDTA (pH 6.5).

An alternative interpretation is suggested by the work of Mitchell and Reith⁸ who discovered that as plants began to senesce the lead they contained was mobilized, substantially increasing in concentration in the shoot. But the pasture in which their study was conducted, unlike that reported here, was a perennial association and the plants were still viable in October when the increase occurred. Subsequent lack of growth due to the low winter temperatures, and the continued translocation of lead may have caused the late autumn and winter increase. The onset of spring growth would have then diluted the lead concentration. My own greenhouse experiments with *A. fatua*, and other reports^{9,10}, suggest that very little lead is translocated in representative grasses of the Benicia-Vallejo area, so that such an explanation for the increase there is unlikely.

A final alternative could be that the increase resulted from a substantial reduction in dry matter of the plant, with no loss of the associated lead. To account for the observed increase, however, a dry matter loss of at least 95% would be required but only a 10–20% dry weight loss actually occurred. This suggests that there is indeed a continuous input of lead into the system, presumably from the atmosphere, much of which is actively retained by plant material. Acting as a store in this way the plants may serve to regulate, at least partially, the cycling of lead within the system.

If the accumulation of lead results from the settling of superficial dust and atmospheric particulates on the surface of the vegetation, it might be expected that with the onset of the winter rains, significant quantities would be removed. In fact, the loss of only small amounts was detected. To investigate the effect of rain, a series of leaching experiments was conducted on dried straw collected during November.

The material was ground to pass through a 20-mesh screen, and 0.1 g samples were placed in flasks with 25 ml. of either H₂O, 0.01 N HCl, or 0.01 M NH₄-EDTA (pH 6.5) for periods of 4 and 24 h. The material was then filtered, washed several times and the leachates were combined. Both the residual material and the leachate were analysed for lead. The results of these experiments are presented in Fig. 2. The EDTA

treatment was very efficient in removing lead, the acid less so, and the water leaching removed very little lead from the plant tissue. These data indicate the tenacity with which lead is retained by the dried plant material and to some extent confirm that rainfall can be expected to be ineffective in removing it.

The accumulation on the surface of the vegetation during the summer, therefore, is possibly followed by a binding of the lead by the dried plants. As very little rain falls in the area during the summer, only negligible amounts of surface lead would be removed, and there would be an extended period of contact during which such binding could take place. Straw consists almost entirely of cell wall remnants and heavy metals are known to be bound by such tissues in both roots and leaves^{11,12}. Analyses of the surface litter reveal lead concentrations of 700–1,000 p.p.m., indicating a further concentrating effect.

The phenomenon described here has important implications for management and surveying in contaminated areas. The California Department of Public Health, in cooperation with the Air Resources Board, has carried out a comprehensive survey of lead in the Benicia-Vallejo region by sampling *A. fatua* over a large area. Because of the propensity of the grass to accumulate lead throughout the season, this may be a suitable method for monitoring the atmospheric input on a large scale. Management could be instituted to reduce the intake by stock of the previous season's contaminated plants. Because only small amounts of lead seem to be taken up from the soil^{9,10}, residual old growth could be ploughed in. Grazing practices might also be altered to avoid the use of contaminated pastures until most of the total feed available is represented by the current season's growth which contains only low concentrations of lead.

I thank Hector Schoo, Shirley Kunisaki and Catherine Rains for technical assistance, and Sydney Thornton and Dr R. G. Burau for their contributions.

D. W. RAINS

Department of Agronomy and Range Science,
University of California,
Davis,
California 95616

Received April 13, 1971.

- 1 Cannon, H. L., and Bowles, J. M., *Science*, **137**, 765 (1962).
- 2 Chow, T. J., and Earl, J. L., *Science*, **169**, 577 (1970).
- 3 Hammond, P. B., and Aronson, A. L., *Ann. NY Acad. Sci.*, **111**, 595 (1964).
- 4 Motto, H. L., Haines, R. H., Chilko, D. M., and Motto, C. K., *Environ. Sci. Tech.*, **4**, 231 (1970).
- 5 Patterson, C. C., *Arch. Environ. Health*, **2**, 344 (1965).
- 6 Haring, C. M., and Meyer, K. F., *Bureau of Mines Bull.*, **98** (1915).
- 7 Smith, R. G., Zajnar, J. S., and Hecker, L., *Environ. Sci. Tech.*, **4**, 333 (1970).
- 8 Mitchell, R. L., and Reith, J. W. S., *J. Sci. Food Agric.*, **17**, 437 (1966).
- 9 Keaton, C. M., *Soil Sci.*, **43**, 401 (1937).
- 10 Marten, G. C., and Hammond, P. B., *Agron. J.*, **58**, 553 (1966).
- 11 Peterson, P. J., *J. Exp. Bot.*, **20**, 863 (1969).
- 12 Reilly, C., Rowel, J., and Stone, J., *New Phytol.*, **69**, 993 (1970).

Effect of an RNA-rich Extract on Acquisition of a One-way Avoidance Response in Rats

Two recent approaches to the biochemistry of memory have involved, respectively, the measurement of molecular changes after training, and attempts, using brain extracts, to transfer

trained behavioural tendencies between animals. Zemp *et al.*¹ have reported differences between experimental and yoked-control mice in the synthesis of both nuclear and cytoplasmic RNA after a training session in which they learned to jump onto a platform to avoid foot shock; subsequent work² suggested that the change in RNA synthesis during learning is a function of the learning experience and not of stimulation, sensitization or other non-specific factors. The "memory transfer" effect was discovered in our laboratory and has now been replicated successfully in at least twenty others³⁻⁵. We had now attempted to interrelate these two approaches by studying the behavioural significance of substances synthesized after acquisition of the same response used by Zemp *et al.*, as measured by their effects when injected into a second animal.

RNA-rich extracts were obtained from the brains of trained animals, untrained controls, and yoked-shock controls (that is, animals receiving the same shocks as trained donors but prevented from learning the avoidance response). Animals receiving injections of extract from these three sources were compared on rate of acquisition of a jump-up avoidance task.

Forty-three 100-150 day old, male albino rats were housed in individual cages and given food and water freely throughout the experiment. An automated one-way avoidance chamber⁶ was used to train animals to avoid a 0.6 mA shock by jumping onto a platform. The conditioned stimulus was the retreat of a wall that had barred access to the platform. Shock followed 10 s later. Thirty seconds after shock onset the wall returned, pushing the rat onto the grid for another trial. A correct trial was scored if the rat jumped to safety before shock onset.

Experimental donors were trained intensively to establish the learning experience firmly. They received ten trials a day for 5 days. Recipients, on the other hand, received three trials a day for 6 days. The difference in procedure reflects the fact that animals receiving ten trials a day mastered the task readily, often reaching asymptotic performance on the second day. Limiting practice to three trials a day made acquisition of the avoidance response more difficult for recipient animals, so that enhanced acquisition could more easily be measured.

The yoked-shock donors were placed in the apparatus for the same time as the experimental donors. Each yoked-shock donor received the same number, duration, and spacing of shocks as an experimental donor; but the 'Plexiglass' wall was always advanced to prevent any learning of escape or avoidance responses.

All rats were handled for about five minutes each day for 10 days before the first day of training. Fifteen of the rats served as donor animals and were assigned on a random basis to an experimental ($N=5$), control ($N=6$), or yoked-shock control ($N=3$) group. One animal was used for chemical assay of the donor material; the remainder of the animals were assigned to three corresponding recipient groups. Members of one group ($N=10$) received extract from the pooled brains of experimental animals. Members of a second group ($N=12$) received pooled extract from the brains of the control group. Members of a third group ($N=6$) received pooled extract from the yoked-shock group.

Within one hour of the last training session, donors were decapitated, and their brains, excluding cerebellum and olfactory lobes, were removed to a beaker of dry ice, where they were kept at -20°C . RNA was obtained by cold phenol extraction⁷.

Twenty to twenty-six hours before its first post-injection test session, each recipient animal was injected intracranially with 0.15 ml. (0.5 brain equivalent) of extract from a trained or untrained donor⁸.

The mean number of avoidances during the eighteen trials was 6.25 for animals receiving control group RNA, 5.83 for animals receiving yoked-shock control group RNA, and 10.5 for animals receiving experimental group extract. Individual rats receiving control group material ranged from four to nine avoidances, while rats receiving experimental material ranged from nine to thirteen. These individual scores were ranked and

submitted to a Mann-Whitney U test. The resulting statistic was highly reliable ($U=0$, $P<0.001$). Yoked-shock recipients ranged from three to ten avoidances. When these scores were ranked against the experimental recipient scores, the resulting test statistic was again highly significant ($U=3$, $P=0.001$). When the yoked-shock and control scores were ranked against each other, however, the resulting statistic was not significant ($U=26$). Fig. 1 presents the mean number of avoidances on each of the training days. As can be seen, the group receiving "trained" extract displayed higher performance on every day after the second.

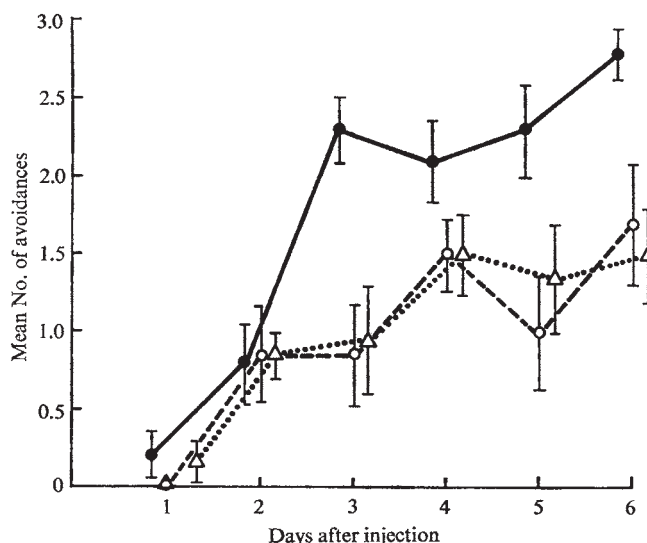


Fig. 1 Mean avoidances of experimental, control, and yoked-shock recipient groups on each of 6 days following injection of RNA-rich brain extract. (The vertical bars at each data point represent one standard error of the mean.) ●—●, Experimental; ○---○, yoked-shock; △---△, control.

The enhancement of avoidance by extracts from trained donor brains is open to several interpretations. The chemical changes in trained animals responsible for this effect might reflect learning, or they simply might reflect general stimulation such as shock trauma, violent activity, or mere exposure to the experimental apparatus. The total ineffectiveness of extracts from yoked-shock donors argues against the latter conclusion. But we did not control for all conceivable sources of stimulation, such as being pushed off the platform. It should also be noted that the cold phenol extract contained many impurities⁷, in addition to RNA. Thus it is not certain that the enhancement was due entirely to RNA.

DAVID H. MALIN
ARNOLD M. GOLUB*
JAMES V. MCCONNELL

*Mental Health Research Institute,
University of Michigan,
Ann Arbor, Michigan*

Received July 12, 1971.

* Present address: Eunice Kennedy Shriver Center, 200 Trepelo Road, Waltham, Massachusetts 02154.

¹ Zemp, J. W., Wilson, J. E., Schlesinger, K., Boggan, W. O., and Glassman, E., *Proc. US Nat. Acad. Sci.*, **55**, 1423 (1966).

² Adair, Linda B., Wilson, J. E., and Glassman, E., *Proc. US Nat. Acad. Sci.*, **61**, 917 (1968).

³ Jacobson, A. L., *J. Biol. Psychol.*, **9**, 52 (1967).

⁴ Tunkl, Judy, *J. Biol. Psychol.*, **19**, 80 (1968).

⁵ Schutjer, Marlys, *J. Biol. Psychol.*, **10**, 54 (1968).

⁶ Tenen, S. S., *Psychon. Sci.*, **6**, 407 (1966).

⁷ Golub, A. M., Masiaz, F. R., Villars, T., and McConnell, J. V., *Science*, **168**, 392 (1970).

⁸ Lindberg, O., and Ernster, L., *J. Biochem.*, **46**, 43 (1950).

BOOK REVIEWS

Pioneers

Sex, Career and Family. By Michal P. Fogarty, Rhona Rapoport and Robert N. Rapoport. (George Allen and Unwin: London, 1971.)

THE initial research of Rapoport, a sociologist-psychanalyst, Rapoport, a social anthropologist, and Fogarty, an economist, aimed to understand the problems of women in top jobs. However, as the work progressed it became clear to them that one could no longer look at women and work as isolated variables. Rather, as their book *Sex, Career and Family* has so convincingly demonstrated, work patterns and family relationships must be re-examined together in the light of changing social mores, aspirations, motivations and opportunities. The single-role stereotypes—the father as provider, the mother as child-raiser-homemaker—have changed. With each passing decade the number of women who work increases, with the greatest increase being among educated women. The more education a woman has, the more likely she is to be employed. For example, in the United States more than 70 per cent of women who have completed more than five years of college are in the labour force. But when we ask how successful they have been in the work they are doing, the picture darkens; this book demonstrates dishearteningly that unless society is willing to undertake major changes in the way jobs are defined, family roles assigned and attitudes challenged, we shall continue to send a double message to young women—"Aim high, but not too high". Given the same intellectual endowment and educational achievement as the men with whom she attended university, the young woman can look forward to a position somewhere in the second rank of any given profession or organization. The world abounds with women whose titles are prefaced by "Assistant".

The authors make it clear that to understand how this down-grading happens to women one must shift emphasis from women alone to men and women

together. From there a short step leads to the exploration of society's legitimization of the relationships between men and women, the family and work. The authors consider family patterns and sex roles as they affect job and career potential for women and, at the same time, open up the crucial psychological issues of self-definition and interpersonal expectations. All of these are examined in the context of changing attitudes toward work and the impact of industrialization and urbanization on social and psychological processes. This matrix of interlocking variables necessarily complicates methodological structure although the authors' struggle to achieve a sensible research methodology is one of the book's important contributions.

The men and women who work out new patterns of relationship between work and family to facilitate the wife's pursuit of an effective career and still maintain a family are pioneers in several dimensions simultaneously. To break the lockstep of man breadwinner and woman homemaker which is so accepted by our social institutions, both the women and men (the dual career families) involved must also change their view of women as the weaker partners. This "creative minority", as the authors call them, are distinctly on the move, spurred by unanticipated social and technological changes.

A reason for this book's excellence, besides the vast spectrum of data drawn upon, is its awareness that one cannot blueprint how or what will be the result of a social, psychological or technological change. Events that seem unconnected emerge as forces to be integrated by these pioneers into the different family, work and personal patterns they definitely predict. As one surveys the rapidity of change in our society, it is hard to fault their insight. Who could have predicted a few years ago the almost universal militancy for equal rights of underprivileged groups which now affects the women's rights movement so directly? Or, as more cogent examples, who could anticipate how definitely one discrete technological advance could change sexual

mores and another the roots of sexual identity?

It is unlikely that the puritanical Henry Ford ever thought he would be the father of America's sexual revolution. His interest was in transportation—mechanical not emotional. Yet he provided America's first bedrooms on wheels, thereby changing sexual behaviour to a greater extent than we can measure. It was a technological achievement external to individuals which began by changing social vistas and eventually reshaped psychological responses.

Now we are in a new era . . . that of the pill. Like Henry Ford, the steroid chemists (were they all men?) were attempting to solve a technological problem . . . how to provide a better method of contraception. To a large extent they have solved the technical challenge . . . for millions of women the pill is a miracle. What was unanticipated and as yet only dimly perceived, are its social-psychological side-effects. Chemists set out to develop safe, cheap, readily available contraception. Once the means to that end came into being, it turned out that the achievement of this seemingly straightforward goal necessitated broad revisions of society's attitudes toward such specific matters as contraception, conception, copulation and consent. On a more abstract level but in the long run probably more salient in determining our attitudes and behaviour will be the revision of our sexual identity.

For example, in any sexual encounter before the pill, the woman had to be protected. Concrete evidence for this assumption is provided in the name of a proto-diaphragm patented as early as 1846 . . . "The wife's Protector". She could protect herself from the spectre of unwanted pregnancy, could ask the man to or could expect it of him as (to drift into the Victorian for a moment) a gentleman. Now the pill is taken along with vitamins, orange juice and morning coffee, separate from and unrelated to the act of going to bed or to the person she might be sleeping with.

The pre-pill perception of the woman was in part that she was weak, fragile

and vulnerable. Can anyone imagine that this persistent image of a woman in this most basic act does not influence men and women's view of themselves and others? If the idea that she must be cared for becomes integrated as part of her sense of self, it also implies that she must be protected from the man who might be careless, that is potentially harmful. As the impregnators, men develop from the same experience similar views of themselves and women. The interaction of these intrinsic sexual identities reinforce each other and the weak-strong dichotomy is maintained. Hence, attitudes derived from the sexual act can affect the way in which children are treated from birth; they can be extrapolated to a general equation describing men and women's roles and functions. She requires protection and pays for it in status. But now she no longer needs the protection—she has it. One can see how in a society pressing towards egalitarianism, the pill would have a definitive impact on that social and psychological equation.

The above oversimplified analysis of the potential impact of the pill on sex, career and family life would probably arouse little disagreement at the nearest cocktail party or at the course, *Women in Society*, recently spawned at the local university. Does that mean we are adapting psychologically and sociologically to the coming change?

Not necessarily. The authors show how little prepared we are individually or as a social group to cope with change despite our awareness that it must come.

Their studies of highly qualified British women graduates of 1960 show that only one per cent of those now married with children definitely intend not to work and that four out of five intend to be in part or full-time work when their children are grown. These findings are similar to those in the United States, where the Labor Bureau reports a marked upswing in the percentage of young women college graduates continuing to work after the birth of their first child. Some of the technical problems associated with this are obvious. How much should be spent on "improved housing, neighbourhood service and provision of nursery schools?" Less obvious are the challenges faced by employers. If it is necessary to re-cast their recruitment and promotion practises to take into account women's different life-cycles, can the community be expected to meet some of the costs coming out of the adaptations which will pay for maternity leaves and relaunching programmes? The authors present evidence that "these programmes can be operated only if there is broad social support from the community and its formal representatives, the law and the govern-

ment". Industry cannot afford and has little inclination for pioneering. This broad social acceptance and support is necessary at all levels if women are not just to be allowed in as assistants but are to reach their capacity at the top of their professions. For, although many women plan to work, the most depressing statistic presented is how few women go far or rise high whether it be in Great Britain, Eastern Europe or the United States.

Thus the change throughout must be in the potential not simply for acceptance but for status: from aspiring for auxiliary roles in work, protection in sexual encounters, or help in the house to expecting an equal opportunity to be leader, sex partner, or non-housekeeper if and when they so choose. This book offers an informed, scholarly approach to new intra-familial relationships which, while not for everyone, would offer a positive direction for families to move in—all within the definition of the family.

DOROTHY S. ZINBERG
NORMAN E. ZINBERG

Men at War

War and the Human Race. Edited by Maurice N. Walsh. (University of California, Los Angeles, Faculty Lecture Series, 1968.) Pp. 95. (Elsevier: Amsterdam, London and New York, 1971.) £2.25.

The editor claims for this small volume that it represents an inter-disciplinary approach to the problem of war. Unfortunately it does not. The fact that the contributors are drawn from six different disciplines, and that no attempt was made to integrate their conclusions—not even in the "Summary and Conclusions"—scarcely justifies the editor's interdisciplinary claim for it. Dr Maurice Walsh, the editor, is a practising psychoanalyst with a gift for misquotation even to the misspelling of common proper names, and a style so banal that it is calculated to confer mediocrity on the most promising of subjects. The book would have benefited from the omission of his "Editorial Introduction", his lecture, "Psychic Factors in the Causation of Recurrent Mass Homicide", and his "Summary and Conclusions", all of which repeat one another, and the principal theme of which is a "new" theory of war.

The theory is that since wars take place at an average interval of 19.6 years, which is the time of the psychic stresses of late adolescence, the period without voting rights and susceptibility to the aggressive propaganda of charismatic leaders, coupled with the intense murderous hostility of the older for the younger males (oedipus complex), the result is recurrent mass homicide (war).

As a true believer Dr Walsh has a properly idolatrous regard for the catchwords of psychoanalysis, which is not, in the end, helpful. We must, however, be grateful to him for the passionate plea he makes for more research, and for having gathered together these interesting lectures.

Dr Bernard Brodie, a political scientist, discusses "Theories on the Causes of War", illuminatingly disposing of the usual theories as to the causes of war, such as munitions makers, big business, innate aggression, arms races, neurotic leaders, and the like. Herbert Friedmann, a biologist, in "Animal Aggression and Its Implications For Human Behaviour", clarifies some of the confused issues in which the term "aggression" is used, carefully distinguishing between the "aggressive" behaviour of other animals and that of man, a very necessary distinction because it has become popular, as Dennis Chitty has recently remarked, to blame our animal ancestors for some of the distressing things we do to each other. Regrettably, when Professor Friedmann writes that man "has innate aggressive tendencies", the statement remains declarative, and no evidence is provided for it.

Dr John G. Kennedy, an anthropologist, writes on "Ritual and Intergroup Murder: Comments on War, Primitive and Modern". This is an excellent contribution and provides a sound overview of conflict and restraint among nonliterate peoples, as well as a fine analysis of their causes, functions, and meaning.

The historian, Jere Clemens King, offers a brief survey of "The Role of Warfare in History", and a journalist, Walter Wilcox, in "The Loneliness of the Long Distance Soldier", provides a first-hand examination of what makes the infantryman run, usually, in the direction in which he is ordered.

ASHLEY MONTAGU

Pesticide Journal

Pesticide Biochemistry and Physiology: An International Journal. Edited by R. D. O'Brien. Volume 1, Number 1. Pp. 122. (Academic: New York and London, March 1971.) \$30.00.

THE most important question to be directed at a new scientific journal is the quality and rigour of the editorial scrutiny that will be applied to the papers proffered. No one would question the capacity of the editorial board listed in this journal: it is to be hoped that they will have the time to spare for the work.

In the first issue four of the eleven papers are from laboratories of members of the board. In general the standard attained is good. However, one paper on resistance in fungi is not

related to the effects of a pesticide. The others include four papers dealing with metabolism by insect gut or microsome preparations. The mode of action of a fungicide on a fungus is described in biochemical terms and that of dieldrin on insects in neurophysiological terms. The metabolism of insecticides in resistant insects is referred to in two papers, one of which suggests that the resistant insect metabolizes one of the new "low toxicity" organophosphorus insecticides like the insensitive mammal. The binding of anticholinesterases to prevent access of meotine or decamethonium to sensitive sites is the subject of another paper. To the reader used to mammals, perhaps the greatest surprise is the explanation of why *Rhodnius* "wets its pants" when exposed to some insecticides.

If this journal can attract high quality papers describing work on the comparative effects of pesticides on target and non-target species it will provide useful background information for the eventual design of more selective but effective pesticides.

For journals whose contents cross the lines of several basic disciplines the titles of the papers are particularly important to enable the appropriate abstracting services to bring them to the attention of others. In this respect this issue is good.

It will be interesting to review this journal again in two or three years time to see whether the editorial aspirations have been maintained. By then one may probably anticipate that the price will have risen above the present £3 for 120 pages and library committees will probably still be looking critically at their budgets. J. M. BARNES

Pharmacological Methods

Methods in Pharmacology. Vol. 1. Edited by Arnold Schwartz. Pp. xiv + 585. (Appleton Century Croft: New York, January 1971.) \$26.50.

THE title of this book is rather misleading as are the headings of many of the chapters. For example, it is surprising to find that a chapter entitled "The Use of Microelectrode Techniques in Muscle" is concerned with microelectrodes and oxygen electrodes in cardiac muscle, not skeletal or nonstriated muscle. In fact, the book concentrates heavily on cardiovascular pharmacology, the remaining chapters being less coherent.

The sections also vary between comprehensive and useful accounts of techniques, such as those on ATPase and on the sarcoplasmic reticulum, to more restricted accounts of personal research having less general interest. The overall effect is that while much of the

volume has interest for the experimental pharmacologist, it can hardly become the place to turn to for a comprehensive account of pharmacological methods. For this purpose the well known texts edited by Laurence and Bacharach and by Siegler and Moyer remain unrivalled. A. S. V. BURGEN

Phase Transitions

Phase Transitions. (Proceedings of the Fourteenth Conference on Chemistry at the University of Brussels, May 1969.) Pp. xi + 256. (Wiley-Interscience: London and New York, March 1971.) £6.

IN 1952 there was an international conference in Paris on *Changements de Phases*. In 1969 the same title was chosen for the Solvay Conference in Brussels, although in the published proceedings this has been translated into English. At least ten people (including myself) took part in both meetings and to us, at least, it is instructive to see how far we have come in seventeen years.

At both meetings the emphasis was on theory. The only exact result known in 1952 was Onsager's recently obtained solution of the two dimensional Ising model in zero field. There is, alas, little more to report now. There are a few further results, notably those of E. Lieb, who reviews this topic, but these have not been so fruitful a source of inspiration as that of Onsager. On the other hand, we have now an immense amount of numerical information on both two and three dimensional lattices. This has been obtained in two principal ways.

First, we have series expansions of the partition function, usually in powers of $1/kT$, and the examinations of these series for singularities by the method of Padé approximants. The start of this work was described in 1952 by Rushbrooke; its most extensive practitioners have been Domb and his group in London, but this work was not described at Brussels.

Second, computers have arrived. These can be used to undertake calculations impossible in 1952, as, for example, the use of the matrix method for semi-infinite lattices, described here by A. Bellemans. Their greatest success, however, has been to act as a "laboratory" in which experiments on simple continuum models can be carried out by Monte Carlo or molecular dynamic methods. Virtually all we know about the melting of a system of hard spheres or about the whereabouts of the critical point of a Lennard-Jones system comes from computer simulation. L. Verlet describes this work. Our purely theoretical knowledge of continuum systems remains in a primitive state, although both J. E. Mayer and S. A. Rice sketch possible lines of attack.

The critical point is always with us.

By 1952 the classical description of van der Waals was no longer accepted (at least by chemists) but there was no agreement about what should take its place. Now the nature of the singularities in the thermodynamic functions is known, if not understood, and much of the interest has passed to the less easily measured transport properties. G. B. Benedek and P. Résibois describe recent progress which has been aided by the advent of the laser, which allows us to study the spectrum as well as the intensity of the scattered light.

The remaining papers delivered at Brussels dealt with more complex systems where experiment rightly still takes precedence over theory. A. R. Ubbelohde spoke on melting and crystal structure (and was also chairman of the conference), G. Busch on liquid metals and S. Lifson on transitions in chain molecules. This welcome book contains also the discussion; some of it rather elliptic, but it may contain as many seeds of future advances as the formal papers. J. S. ROWLINSON

Band Theory

The Electron Band Theory of Solids. By G. C. Fletcher. Pp. xv + 260. (North Holland: Amsterdam and London, 1971.) £6.30.

THERE are already a number of advanced texts devoted to the electron band theory of solids, but for some time there has been a need for a treatment of this particular specialization from first principles, that carries the subject to a level from where the reader can easily tackle the advanced literature, which deals with the technicalities involved in actual band theory calculations. This book adequately fulfils this need.

The basic principles of the subject are introduced, in the first few chapters, in a way that should be easily understood by the average graduate, without demanding a wide command of mathematical skills (several later chapters are devoted to mathematical techniques), yet without recourse to tedious methods nor with loss of rigour. In this section of the book the author very briefly discusses the validity of the one-electron treatment, although this is dealt with in much greater detail in the appendix. Crystal and reciprocal lattices are discussed adequately, and the appendix compliments these topics by providing a useful set of figures illustrating the Bravais lattice types and corresponding Wigner cells and Brillouin zones. Density of states and related matters are dealt with briefly, and a chapter is devoted to the problems of choice of realistic initial potentials, discussion being confined to those of "muffin-tin" type. Discussion of the exchange term occurring in these potentials is relegated

to the appendix. Two chapters dealing with the free and nearly free electron approximations conclude what I would regard as the introductory section of this book. These two topics are used to provide useful illustrations of the restricted and unrestricted zone schemes, and of the formation of energy gaps.

A considerable portion of the book is devoted to an introductory review of some of the more important methods adopted for solving the one-electron equation. These methods are returned to, a few chapters later on, after ideas of symmetry and degeneracy have been discussed and it is then shown how the methods of group theory can be used to facilitate the obtaining of solutions at points of high symmetry. A chapter deals briefly with relativistic effects, too briefly I suspect, for most readers at whom the book is aimed and consultation of works listed in the reference section at the end of the chapter will probably be necessary. Other topics considered in the remaining chapters include the pseudopotential method and various semi-empirical and interpolation techniques.

An extensive appendix is provided and apart from items already mentioned, topics include the principles of numerical integration, Legendre polynomials and other mathematical functions encountered in the main text, and Lowdin's expansion. There is additional work filling in on the Green's function method and proofs connected with the theory of group representations.

In conclusion, this is a generally well-written book with its subject matter crisply presented. It should not be omitted from lists of recommended texts for postgraduate courses in solid state physics and metallurgy. It clearly fulfils the author's avowed aim to provide a primer for people wishing to carry out actual band theory calculations and to give guidance for the assessment of the reliability of such calculations.

M. GUMBRELL

Complex Permittivity

Complex Permittivity: Theory and Measurement. Compiled by B. K. P. Scaife, W. G. S. Scaife, R. G. Bennett and J. H. Calderwood. Pp. vi+170. (English University: London, 1971.) £3.45.

THIS monograph on dielectric materials consists of three separate chapters, the first, by B. K. P. Scaife, is on the theory of dispersion in polar dielectrics, the second, by W. G. S. Scaife, discusses the effects of hydrostatic pressure on the properties of dielectrics, and the final chapter, by J. H.

Calderwood and R. G. Bennett, deals with experimental methods for the measurement of complex permittivity.

The first chapter gives a most readable account of the theory of dispersion. An initial section on the macroscopic approach has the fluctuation-dissipation theorem as the central theme, and a link between microscopic and macroscopic considerations follows in the next section. Details of the microscopic treatment follow, and include inertial and dipole-dipole coupling effects. A new method is described for plotting experimental results when inertial effects are important. This has the advantage that the complex plane plots are still circles, whereas the Cole-Cole ϵ'' against ϵ' plots give more complicated curves when inertia becomes important.

The next chapter, on pressure effects, briefly reviews the theory for non-dipolar fluids, and then describes the experimental techniques which have been used, with some discussion of the results obtained. Sections on crystalline solids and on polar fluids follow; in both the emphasis is on the interpretation of experimental results in the light of theory. There is a final section on dipolar relaxation processes in solids.

The final chapter on experimental techniques gives a clear and straightforward account of the principles and basic theory of a number of different experimental methods, from bridges through resonance methods to waveguides, thus covering a wide range of frequencies. Particular emphasis is placed on sources of error and practical convenience, and the influence of the computer on experimental method has not been overlooked. There are a few blemishes. No suggestion is made, for example, about sub-millimetre wave measurements, yet the first chapter contains a proposed new method for analysing experimental results in this region of the frequency spectrum, so crucial to the inertia effects which occupy so much of the book. There is no mention of resonant cavity techniques, yet the transformer ratio-arm bridge is described separately in chapters two and three.

This book will undoubtedly be useful to specialists in this field, and can be recommended. It must be understood, however, that it is a collection of three related review articles, rather than a comprehensive treatment of all aspects of dielectric materials.

A. L. CULLEN

Lasers Applied

Lasers and Their Applications. By M. J. Beesley. Pp. xii+234. (Taylor and Francis: London, July 1971.) £5.

THE author's stated aim in writing this book is "to explain the mechanism of

laser action . . . and to describe some applications of the laser . . .", but it is not clear, on reading the book, to whom it is directed.

The level of the material included is highly variable—for example, it assumes that the reader will need help with the most elementary algebra but that a casual reference to free-space mode densities suffices. The initial parts of the book suffer from too extreme condensation, which has resulted in many half truths or misleading statements—for example, "for most atoms and molecules . . . the first level above (the ground state) is separated by a gap of 1.25 eV". Fortunately this does not hold for the ammonia molecule, description of which follows the above rather sweeping comment. From the text it may appear that the inversion mode is the only possible vibration mode for NH_3 .

The description given of the beam focuser supplies a lot of irrelevant detail but nowhere describes why or how the system works. It is implied that the amplitude of a lightbeam reflected at a plane surface at non-normal incidence is independent of the state of polarization of the light. One can unfortunately find all too many examples of this kind of obscurity and matters are not always helped by carelessness in the exposition. (Thus the onus is on the reader to deduce whether the expression "direction of plane-polarized light" refers to propagation or electric vector.)

The author's practical experience with lasers is reflected in the brief descriptions of laboratory procedures. These parts of the book are more successful and really it looks as though the author has written the wrong book. The average scientific paper tends to contain a simple diagram showing arrays of, for example, mirrors or other optical devices. It is not uncommon for newcomers to the field to have to spend a lot of time gaining experience in this area and the ideal solution—to work under the guidance of an "old hand"—is not always possible. (This is of particular relevance in the laser field, where applications are possible in areas where no optical experience exists.) It does seem that, if the author had addressed himself specifically to this problem, a far more satisfactory result could have been achieved.

The selection of applications chosen for description is well made and gives an excellent view of the potentialities of the laser in a variety of fields. Unfortunately, as in the earlier part of the book, some of the descriptions lack the clarity required for the unfamiliar reader. Sufficient references allow further exploration of the topics described.

O. S. HEAVENS

CORRESPONDENCE

Six Years for a PhD

SIR,—The impression exists among many students of college age and earlier, and among their families, that three years is a “normal” length of post-baccalaureate time for a PhD. Many university faculty expect their graduate students to finish in four, or perhaps five, years. Those who

case of foreign students, it was not always a BA or BS. No adjustments were made for interrupted or part-time study. Obviously most of those taking over ten years were not in graduate full-time for the entire period. The curve for mid-year degrees, which at Harvard are dated “March”, is shifted left by six months to provide a better fit.

gradually extending out to approximately 30 years. The number who receive PhDs in the traditional three years or less is negligible: less than one per cent.

The curves for mid-year and June degrees agree also in absolute numbers for the wings; additional recipients of June degrees are clustered at 5 and 6 years. The sharp drop thereafter probably represents the effect of the existence of deadlines, not only departmental rules but also psychological pressure felt by the individuals, both students and advisers.

The California Institute of Technology is a more specialized institution, and the 117 PhDs awarded in June 1971 were all in scientific subjects. Yet the distribution (Fig. 2) is very similar to that for all Harvard degrees, peaking at 5 and 6 years with a long tail that extends out to 16 years.

The average length of time of post first-degree enrolment before receiving a Caltech PhD is 6.4 years, with a median of 6 years and a mode of 6 years.

Clearly, the fact that so many students take more than five years for a PhD reflects more than individual delays, and must result partly from the design of

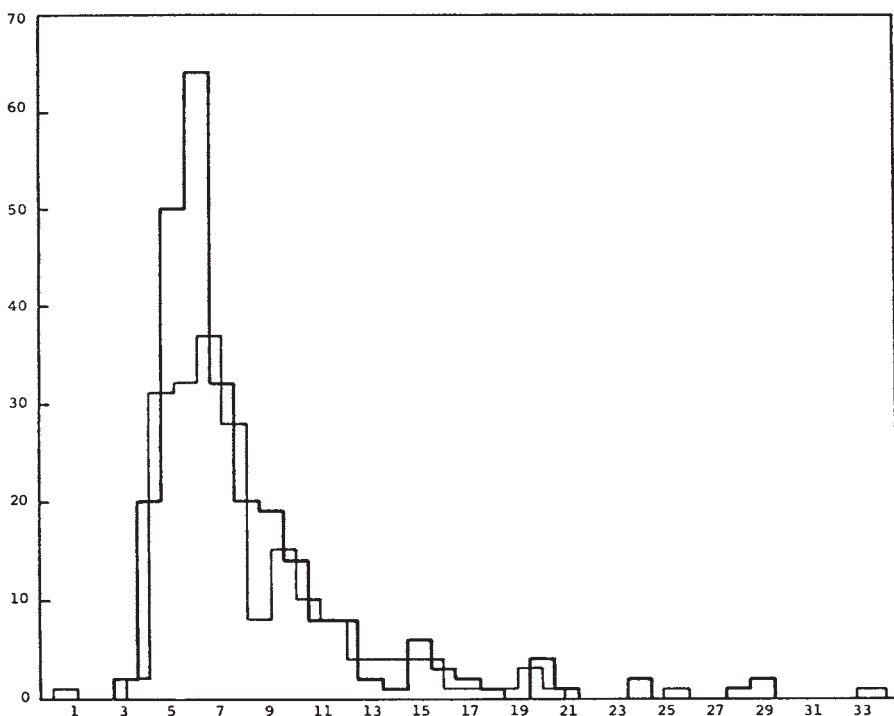


Fig. 1 Number of years between first degree and PhD for those receiving degrees at Harvard commencement in 1969. The dark line is June degrees; the light line is March (mid-year) degrees.

spend six years or more in graduate study are often asked why they took so long. As we shall see here, however, six years is about the median time for a PhD at both Harvard and Caltech.

Fig. 1 shows the distribution of time between first degree and doctorate for all students receiving PhDs at Harvard University in 1969. The first degree is always used although, especially in the

The curves, which are drawn to the same absolute scale, agree in the form of the distribution, which peaks at five through seven years. The average length of time spent before receiving the 262 June 1969 degrees at Harvard was 7.9 years, with a median of 6 years and a mode of 6 years. The average for the 459 total degrees was 8.9 years, with a median of 7 years. There is a long tail,

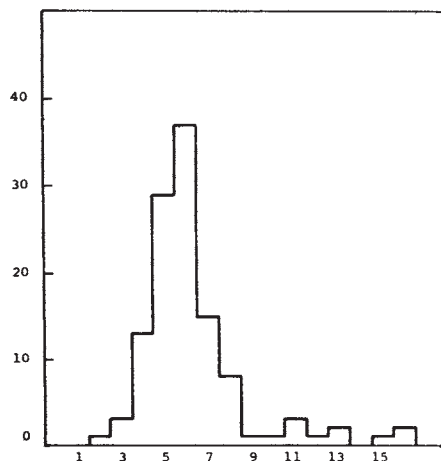


Fig. 2 Number of years between first degree and PhD for those receiving degree at California Institute of Technology commencement in 1971.

requirements laid down by the departments, including minor theses, qualifying examinations, and supplemental research projects.

Yours faithfully,

JAY M. PASACHOFF

*Hale Observatories,
Carnegie Institution of Washington,
California Institute of Technology,
Pasadena, California 91109*

Bird Navigation

SIR,—Two points appear to be misleadingly used to indicate that domesticated *Columba livia* does not require sun-azimuth for route-finding when your correspondent on the homing sense in pigeons (*Nature*, 232, 86; 1971) stated: "Recent evidence that this ("map" mind of the homing bird) can operate effectively even after many days' imprisonment away from the home loft . . ." I published elsewhere¹ examples of very long memory in pigeons including the following: a Hollywood (USA) pigeon which raced up to 500 miles, 1931–4, sold to Huntington (California) which returned on escape after kept "prisoner" 8 years; one lost racing Rennes (France) to Manchester, 1926, which returned home 1931, after presumably being stolen and kept prisoner; an Australian pigeon lost racing 550 miles Benallo–Melbourne, 1944, which returned in 1948, and another which returned 400 miles to France 2 years after sale to Newcastle, where it was kept prisoner a year. I have known pigeons lost up to a year return to their old nest-boxes, among many inside a breeding loft, and fight the new owner for occupation. As the breeding season approaches, more lost birds home than otherwise.

In all such cases which could be investigated, the birds were kept as "inside" prisoners without sky-views. Where transported birds are kept prisoner a week or more in outside, mesh-wire "aviaries", they "resettle" to the new territory. Young birds (6 months old) have thus been resettled in 1 week, exceptionally in 4 days; but older birds usually require 8 to 10 days. My operational birds in the British Army Pigeon Service in the 1939–45 war were sent forward in baskets (to be flown back to their lofts) with the instruction "it is important that birds should be so placed that it is impossible for them to see their immediate external surroundings", and that unused birds should not be kept in delivery or stock baskets for more than 4 days (in trench-baskets, 3 days), but returned for replacements. This was to avoid birds "seeing" and "remembering" the new temporary release-point.

Visual recognition of their external surroundings (e.g. sky and landscape

was used to resettle birds to "home" to new loft-sites in place of old ones, using outside aviaries or baskets on top of the loft for at least an hour daily. I allowed my loftmen a minimum of a fortnight to settle pigeons to a new loft-site, but preferred 5 weeks (longer still for distances over 30–50 miles). We once settled thirty-four birds to "home" to a new loft site in 10 days. I have no evidence of birds settled to fly to a new loft-site by "inside" imprisonment, without external views, excepting the "nomad" type of training where a pigeon homed to a marked mobile loft, or basket, by recognition of the mark used also when feeding it.

While ability to home is known to be inherited (an untrained 2-months-old pigeon still squeaking homed 40 miles to one of my lofts, where it was bred) the homing territory is acquired after birth—pigeon eggs taken 100 miles to be hatched and reared "home" to the loft where they were reared, not where the eggs were laid. Pigeons returning to Scotland continued through the longer light of Scottish summer nights to arrive earlier than birds roosting in the darker English night before reaching English lofts. Pigeons liberated in fog distinct from merely ground-mist, are delayed, presumably by waiting for visibility to clear.

The more remarkable test of astral and sun navigation is not this long delayed homing of pigeons, but the annual return to the same breeding and wintering territories by trans-equatorial migrants, particularly the purely marine southern petrels and shearwaters, whose journeys cover seas with no landmarks.

Yours faithfully,

ERIC HARDY

*Merseyside Naturalist's
Association,
47 Woodsorrel Road,
Liverpool 15*

¹ Hardy, E., *Pigeon Guide: A Complete Handbook of Pigeon Keeping* (Seeley, London, 1951).

Black Hole to the North?

SIR,—I read with extreme interest the editorial observation that, over the past ten years, "the population in the extreme North of Britain decreased, with Lancashire, Cumberland and Northumberland all returning figures lower than in 1961 . . ." thus confirming the fears of those of us who dwell beyond the pale in the thirty-one counties of mainland Britain on the wrong side of Hadrian's Wall, that in fact we really do have no existence and that contracting population acting like some contracting stellar body has pulled the land mass with it leaving a "black hole" to the north of Carlisle.

Or perhaps the statement showed a trace of Anglo-Chauvinism in confusing the land of the English with the island of

Great Britain?—an inauspicious error in a journal dedicated to the objective accuracy of science.

Yours faithfully,

WILLIAM F. DRYDEN

*Department of Pharmacology,
Royal College,
George Street, Glasgow C1*

¹ *Nature*, 232, 512 (1971).

Unfair to Scientists

SIR,—During the period when the pay of civil service scientists has been the subject of public debate most of us concerned with the matter have felt the reports in *Nature* to be well balanced and to fairly state the case of both sides. Your conclusions have represented a fair assessment of the merits of the case. But, then, we could expect you as a serious commentator on scientific affairs to recognize what enormous damage would be done to the scientific community at large by the Civil Service Department's ludicrous pay offer to its scientific staffs. I should like, however, to emphasize the following points.

Regrettably, the arbitration tribunal has done nothing to resolve the basic problem. Anyone trying to reconcile the irreconcilable is likely to produce a nonsensical result. That was bound to be the outcome of trying to bring together "fair comparisons" and internal relativities. The Civil Service Department at least recognized the problem. They acknowledged that there were special circumstances affecting the pay of scientists but argued that they were irrelevant and, in any case, could not be measured. Thus, for them, there was no conflict. We were not prepared, and are not now, to have scientists treated as poor relations of their civil service colleagues in the technological field, nor yet of those in administration.

We will not take part in another pay research exercise and we are determined to get a joint review to establish a proper basis for future pay reviews for scientific staffs. We will press for early action on all of the structure aspects of Fulton, including the point which you rightly make about the need for scientists (and other specialists) to move across to more general administration. In part, of course, the point can be met by proper systems of accountable management but there will remain a need to move away from science for those who are going to get right to the top.

Neither this alone, nor in combination with your other point of allowing greater freedom to leave the civil service (a subject on which we are currently in discussion with CSD) will resolve the main problem. This remains the need to provide the great majority of scientific

staffs—who will not become Chief Scientists or Permanent Secretaries—with a pay and career structure which matches that of those of their colleagues whose job weight, background and qualifications is unquestionably in no way superior to their own. That is not the case now; we are quite determined that it shall become so.

All this is far removed from your suggestion that we may become “entirely lost in detailed quibbles”, or that we are taking an insufficiently radical view of what is needed. I should have thought that our aims are precisely those which would result in scientists assuming their rightful position in the Service. As you have recognized, it will be a sorry day for science and scientists generally if we fail. We do not intend to fail; there is too much at stake.

Yours faithfully,

CYRIL COOPER

Deputy General Secretary

*The Institution of Professional Civil Servants,
Northumberland Street,
London WC2*

History of “Plasmas”

SIR,—There has been speculation as to the basis for Irving Langmuir’s use of the word “plasma” to describe a particular

region of an electrical discharge in a gas. I was working for Langmuir when he made this innovation about 1927, and, much later, described it in a letter to a friend at the General Electric Research and Development Center, dated April 20, 1967. The pertinent extract from this letter follows.

“Meanwhile word had come down from on high that we had better do something about mercury-arc rectifiers and converters, which were the big new things in the electrical engineering world, although at that time they were all in glass bulbs. So Langmuir began to study mercury vapor discharges. He shortly invented his probe, I did the experimental work and most of the mathematics, and we soon accumulated a lot of data about ion densities and velocity distributions in the mercury arc columns, in the positive columns of Geissler tubes, and in gas-filled thermionic tubes.

“We noticed the similarity of the discharge structures they revealed. Langmuir pointed out the importance and probable wide bearing of this fact. We struggled to find a name for it. For all members of the team realized that the credit for a discovery goes not to the man who makes it, but to the man who names it. Witness the name of our continent. We tossed around names like ‘uniform discharge’, ‘homogeneous discharge’, ‘equilibrium discharge’; and for the dark or light regions surrounding

electrodes, names like ‘auras’, ‘haloes’, and so forth. But one day Langmuir came in triumphantly and said he had it. He pointed out that the ‘equilibrium’ part of the discharge acted as a sort of sub-stratum carrying particles of special kinds, like high-velocity electrons from thermionic filaments, molecules and ions of gas impurities. This reminds him of of the way blood plasma carries around red and white corpuscles and germs. So he proposed to call our ‘uniform discharge’ a ‘plasma’. Of course we all agreed.

“But then we were in for it. For a long time we were pestered by requests from medical journals for reprints of our articles. This happens to me this day. The scientific world of physics and chemistry looked askance at this uncouth word and were slow to accept it in their vocabulary. The engineering world treated it as a GE trade name. Then all of a sudden, long after I had left the laboratory, to my pleased surprise, everybody started to talk about plasmas. This happened not long before they became thermonuclear and so government subsidized. That finally put the seal of respectability on plasmas.”

Yours faithfully,

HAROLD M. MOTT-SMITH

6909 SW Freeway,
Texas 77036

British Diary

Saturday, September 18

Cell Proliferation and the Differential Response of Normal and Malignant Cells, Professor L. F. Lamerton, British Institute of Radiology, at Southampton. (Silvanus Thompson Memorial Lecture.)

Tuesday, September 21

Infra-red Techniques (three-day conference) Institution of Electrical Engineers; and the Institution of Electronic and Radio Engineers, at the University of Reading.

Synthesis of Sugar Phosphonates, Professor H. Paulsen, Carbohydrate Group (Chemical Society Subject Group), at Birkbeck College, University of London, Malet Street, London WC1.

Wednesday, September 22

Fibre Optics (7.30 p.m.) Dr M. Chown, Institution of Electrical Engineers,

jointly with the Harlow Engineering Society, at Harlow Technical College, Harlow, Essex.

Nuclear and Particle Physics (three-day conference) Institute of Physics and the Physical Society, at the University of Oxford.

On-Line Computer Methods Relevant to Chemical Engineering (7.30 p.m. symposium) Institution of Chemical Engineers, jointly with the British Computer Society, at the University, Nottingham.

Process Pumps (three-day conference) Institution of Mechanical Engineers, at the University of Durham.

Thursday, September 23

MOS Integrated Circuits (7 p.m.) Mr R. G. Hibberd, Institution of Electrical Engineers, at the Medway College of Technology, Chatham, Kent.

Population and Pollution (two-day symposium) Eugenics Society, in the Meeting Rooms of the Zoological Society of London, Regent’s Park, London NW1.

The Consumers’ Association—Its Aims and Achievements (7 p.m.) Mrs Alma Williams, Oil and Colour Chemists’ Association, at the Beech Tree Hotel, Maxwell Road, Beaconsfield.

Friday, September 24

Chlorinated Rubber Marine Paints (6.30 p.m.) Mr C. G. Reid, Oil and Colour Chemists’ Association, Midlands Branch, at the Chamber of Commerce and Industry, 75 Harborne Road, Birmingham.

Some Aspects of Metal Pretreatment and Printing (7.15 p.m.) Mr P. Gollop, Oil and Colour Chemists’ Association, at the Royal Hotel, Bristol.

Saturday, September 25

Science Simulation Seminar, Institute of Mathematics and Its Applications, and Chelsea College of Science and Technology, at the Centre for Science Education, Bridges Place, London SW6.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

- Imperial College of Science and Technology, University of London. Prospectus 1972-73. Pp. 139. (London: Imperial College, 1971.) [36]
- Building Research Station Digest, No. 57: Painting Walls—3. Pp. 8. (London: HMSO, 1971.) 5p. [36]
- Meteorites: a Concise Account. By A. A. Moss. Pp. vii+26. 20p. Bulletin of the British Museum (Natural History). Entomology. Vol. 26, No. 2: The Species of *Ardeicola* (Phthiraptera: Ischnocera) Parasitic on the Ciconiidae. By P. Kumar and B. K. Tandan. Pp. 117-158+2 plates. £1.80. Bulletin of the British Museum (Natural History). Historical. Vol. 4, No. 2: A Short History of the Libraries and List of MSS. and Original Drawings in the British Museum (Natural History). By F. C. Sawyer. Pp. 77-203. £3.40. Type-Specimens of Birds in the British Museum (Natural History). Vol. 2: Passerines. By Rachael L. M. Warren and C. J. O. Harrison. Pp. vi+628. £13. (London: British Museum (Natural History), 1971.) [36]
- Tin Research Institute. Annual Report 1970. Pp. 36. (Greenford, Middx.: Tin Research Institute, 1971.) [46]
- Rothamsted Experimental Station. Report for 1970. Part 1: Pp. 385. Part 2: Pp. 259. (Harpenden, Herts: Rothamsted Experimental Station, 1971.) £2 the two parts. [76]
- Job Enrichment and Employee Participation—a Study. By Margaret Butteriss. Pp. 71. (London: Institute of Personnel Management, 1971.) £1. [76]
- Proceedings of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 323, No. 1553 (8 June 1971): A Discussion on Numerical Analysis of Partial Differential Equations. Organised by J. H. Wilkinson, FRS. Pp. 151-304. £1.75; \$4.55. Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 261, No. 837 (27 May 1971): A Discussion on New Developments in Electron Microscopy with special emphasis on their Application in Biology. Organised by H. E. Huxley, FRS, and A. Klug, FRS. Pp. 1-230+plates 1-44. £9.50; \$24.50. Vol. 261, No. 838 (27 May 1971): On the Upper Triassic Mammals. By F. R. Parrington, FRS. Pp. 231-272+plates 45 and 46. £1.25; \$3.25. (London: Royal Society of London, 1971.) [86]
- At Your Service 1971/1972: An Outline of Advisory and other Services, Grants and Subsidies available to Farmers and Horticulturists. Pp. 40. (Belfast: Ministry of Agriculture, 1971.) [96]

Other Countries

- Institute of Applied Science of Victoria. Report of Activities 1968/1969. Pp. 22. (Melbourne: Institute of Applied Science of Victoria, 1971.) [245]
- Unesco. Science Policy Studies and Documents No. 25: Science Policy and the European States. Pp. 208. (Paris: Unesco; London: HMSO, 1971.) 12 francs; 90p; \$3. [245]
- The American Phytopathological Society. Monograph No. 6: Fusarium Wilt of Tomato. By J. C. Walker. Pp. 56. (St. Paul, Minnesota: The American Phytopathological Society, 1971.) \$3. [245]
- New Zealand: Ministry of Transport. Meteorological Observations for 1967—Stations in New Zealand and Outlying Islands, including the Cook Group. Pp. 68. (Wellington: Government Printer, 1970.) [245]
- Brands, Generics, Prices and Quality: The Prescribing Date After a Decade. Pp. vii+118. (Washington, DC: Pharmaceutical Manufacturers Association, 1971.) gratis. [255]

- Standards in Canada. By Robert F. Leggett. (A study prepared for the Economic Council of Canada and the Science Council of Canada.) Pp. 248. (Ottawa: Information Canada, 1971.) \$3.25. [255]
- US Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. The 1965 Salt-Water Angling Survey. By David G. Deuel and John R. Clark. Pp. 51. (Washington, DC: Government Printing Office, 1968.) \$0.40. [255]
- Ecology and Land Use of the Supply Ponds Natural Area, Branford, Connecticut. By Diana Starr Cooper and William Lee Hotaling. Pp. x+56. (New Haven, Conn.: Yale University, School of Forestry, 1970.) \$2. [255]
- US Department of the Interior: Geological Survey. Water-Supply Paper 1929: Surface Water Supply of the United States 1961-65. Part 2: Pacific Slope Basins in California. Vol. 2: Basins from Arroyo Grande to Oregon State Line, except Central Valley. Pp. ix+673+1 plate. (Washington, DC: Government Printing Office, 1970.) \$3.25. [255]
- Canada: Department of Energy Mines, and Resources. Geological Survey of Canada. Memoir 360: Paleozoic Geology of the Bruce Peninsula Area, Ontario. By B. A. Liberty and T. E. Bolton. Pp. 163. \$4.50. Paper 70-35: Paleozoic Geology of Wilfrid Island, Bath, Sydenham and Gananogue Map-Areas, Ontario. By B. A. Liberty. Pp. 12+4 maps. \$3.50. (Ottawa: Information Canada, 1971.) [255]
- Malaysia. Ministry of Lands and Mines. Annual Report of the Geological Survey Malaysia, 1968. Director: S. K. Ching. Pp. viii+199+20 plates. Sandakan Peninsula, Eastern Sabah, East Malaysia: Explanation of Sheets 6/337/16, 6/118/13, 5/117/4, and 5/118/1. By D. T. C. Lee. (Report No. 6.) Pp. xiii+75+36 plates. M\$12. (Kuching, Sarawak, Malaysia: Principal Geologist, Geological Survey, 1970.) [255]
- World Health Organization. Technical Report Series No. 466: Methodology for Family Studies of Genetic Factors—Report of a WHO Scientific Group. Pp. 37. (Geneva: World Health Organization; London: HMSO, 1971.) 3 Sw. francs; 30p; \$1. [16]
- Nova Scotia Research Foundation. Selected Bibliography on Algae, No. 11. Pp. 169. (Dartmouth, Nova Scotia: Nova Scotia Research Foundation, 1971.) [16]
- Ministerio de Agricultura y Pesqueria, Direccion General de Investigaciones Agropecuarias. Boletín Técnico. No. 72: El Cultivo de los Citricos en el Peru. Pp. 60. No. 73: El Cultivo del Palto en el Peru. Pp. 40. No. 74: El Cultivo del Mango en el Peru. Pp. 39. No. 75: El Cultivo de la Pina en el Peru. Pp. 32. (Lima, Peru: Ministerio de Agricultura y Pesqueria, 1969 and 1970.) [16]
- Smithsonian Contributions to the Earth Sciences. No. 6: Distribution of *Echinarachnius parma* (Lamarck) and Associated Fauna on Sable Island Bank, Southeast Canada. By Daniel J. Stanley and Noel P. James. Pp. 24. \$0.45. Smithsonian Contributions to Zoology. No. 72: Revision of the Fish Genus *Esenius* (Belontiidae, Blenniinae Salariaiini). By Victor G. Springer. Pp. 74. \$1.25. No. 73: Synopsis of the Tribe Salariaiini, with Description of Five New Genera and Three New Species (Pisces: Blenniidae). By William F. Smith-Vaniz and Victor G. Springer. Pp. 72. \$1.25. No. 82: Pontoniid Shrimps from the Ninth Cruise of R/V *Anton Bruun*, 110E, 1964: I. *Palaemonella* Dana and *Periclimenes* Costa. By A. J. Bruce. Pp. 13. \$0.25. No. 83: The Distribution and Patterns of the Major Arteries of the Iguanids and Comments on the Intergeneric Relationships of Iguanids (Reptilia: Lacertilia). By George R. Zug. Pp. 23. \$0.40. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [16]
- US Department of the Interior: Geological Survey. Bulletin 1350: Lexicon of Geologic Names of the United States for 1961/1967. By Grace C. Keroher. Pp. iv+848. \$3.50. Professional Paper 629-A: Upper Paleozoic Rocks in the Quairrh Mountains and Bingham Mining District, Utah. By E. W. Tooker

- and Ralph J. Roberts, et al. Pp. iv+76. \$1. Professional Paper 661: Mississippian Stratigraphy of the Diamond Peak Area, Eureka County, Nevada. By David A. Brew. Pp. iv+84+1 plate. Professional Paper 674: Stratigraphy of the Outcropping Post-Magathy Upper Cretaceous Formations on Southern New Jersey and Northern Delmarva Peninsula, Delaware and Maryland. By James P. Owens, James P. Munard, Norman F. Sohl and James F. Mello. Pp. iv+60. \$0.65. Water-Supply Paper 1899-C: Sediment Transport by Streams in the Palouse River Basin, Washington and Idaho, July 1961/June 1965. By P. R. Boucher. Pp. iv+37+plate 1. \$0.70. (Washington, DC: Government Printing Office, 1970 and 1971.) [16]
- Publikationer fra det Danske Meteorologiske Institut, Isforholdene i de Grønlandske Farvande, (The Ice-Conditions in the Greenland Waters), 1964. Pp. 25+32 Ice-Charts. (Charlottenlund, Danmark: Det Danske Meteorologiske Institut, 1971.) [16]
- US Department of the Interior: Geological Survey. Water-Supply Paper 1932: Surface Water Supply of the United States 1961/65. Part 12: Pacific Slope Basins in Washington. Vol. 1: Pacific Slope Basins in Washington except Columbia River Basin. Pp. ix+679+1 plate. (Washington, DC: Government Printing Office, 1971.) [26]
- Smithsonian Contributions to Zoology. No. 78: A Manual for the Study of Meiofauna. By Neil C. Hulings and John S. Gray. Pp. xi+83. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) \$2.75. [26]
- Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 139: Der Gelbrost, *Picinia Striiformis* West. II. Befallsbild, Morphologie und Biologie der Sporen. Infektion und weitere Entwicklung. Wirkungen auf die Wirtspflanze. Von Professor Kurt Hassebrauk. Pp. 111. 32 M. Heft 140: Beitrag zur Kenntnis der *Phytophthora*-Arten in Iran und ihrer Phytopathologischen Bedeutung. Von Dr. Djafar Ershad. Pp. 84. 33.95 M. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1970 and 1971.) [26]
- Annals of the New York Academy of Sciences. Vol. 171, Article 2: Down's Syndrome (Mongolism). By V. Apgar et al. Pp. 303-688. (New York: New York Academy of Sciences, 1970.) \$24.50. [26]
- Annual Summary of Information on Natural Disasters, No. 4, 1969. Pp. 73. (Paris: Unesco; London: HMSO, 1971.) 10 francs; 75p; \$2.50. [26]
- Recueil de Travaux du Bureau International de Poids et Mesures, Volume 2, 1968-1970, 29 reprints. (Sèvres, France: Bureau International des Poids et Mesures, 1971.) [26]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wheat Research Unit 1969/1970. Pp. 26. (Ryde, NSW: CSIRO, 1971.) [46]
- US Department of the Interior: Geological Survey. Water-Supply Paper 1899-J: Ground Water for Irrigation Near Lake Emily, Pope County, West-Central Minnesota. By Wayne A. Van Voast. Pp. iii+28. (Washington, DC: Government Printing Office, 1971.) \$0.25. [46]
- Smithsonian Contributions to Zoology. No. 68: A Systematic and Ecological Study of Nearctic Hydrilina (Diptera: Ephydriidae). By D. L. Deonier. Pp. 147. \$1.75. No. 79: Western Atlantic Shrimps of the Genus *Metapenaeopsis* (Crustacea: Decapoda, Penaeidae), with Descriptions of Three New Species. By Isabel Pérez Farfante. Pp. 37. \$0.45. No. 94: Type Specimens in the Hans Eggers Collection of Scolytid Beetles (Coleoptera). By William H. Anderson and Donald M. Anderson. Pp. 38. \$0.45. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [46]
- World Meteorological Organization. WMO Sea-Ice Nomenclature: Terminology Codes and Illustrated Glossary. Pp. 147. (Geneva: World Meteorological Organization, 1970.) [46]

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A.	Canada
Nature (Friday)	£14	\$48	\$52
Nature + Nature Physical Science	£24	\$83	\$90
Nature + Nature New Biology	£24	\$83	\$90
All three editions	£29.50	\$108	\$116
Annual Index	£1	\$3	\$3

* Rates for shorter periods *pro rata* (minimum three months)

(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED

4 LITTLE ESSEX STREET, LONDON WC2R 3LF

Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING

WASHINGTON DC 20004

Telephone Number: 202-737 2355

Subscription Department

MACMILLAN JOURNALS LIMITED

BRUNEL ROAD, BASINGSTOKE, HANTS

Telephone Number: Basingstoke 5431

American display advertisements

NATURE SCIENTIFIC PUBLICATIONS INC

711 NATIONAL PRESS BUILDING

WASHINGTON DC 20004

All other advertisements

T. G. SCOTT & SON, LIMITED

1 CLEMENT'S INN, LONDON WC2A 2ED

Telephone: 01-242 6264/01-405 4743

Telegrams: Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, September 17 1971